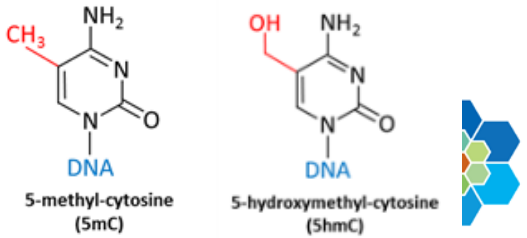
Epigenetics: Normality in Toxicologically Relevant Species:

Development of a framework to better understand the impact of epigenetics on (eco)toxicology.

Professor Richard R Meehan Nov 17th Brussels

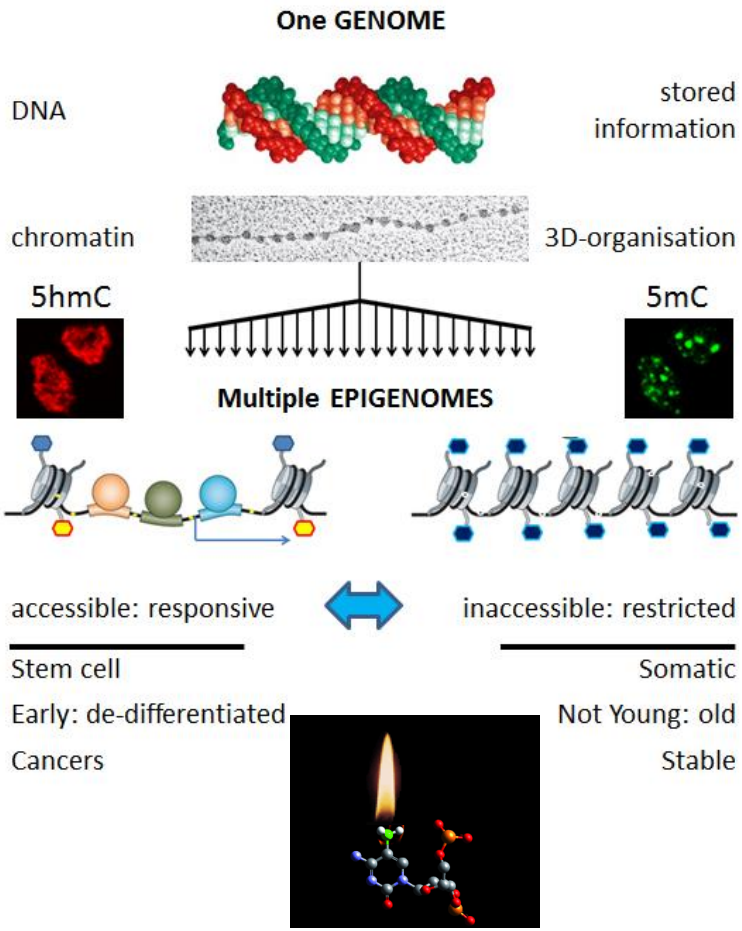
www.hgu.mrc.ac.uk/people/r.meehan_researchb.html

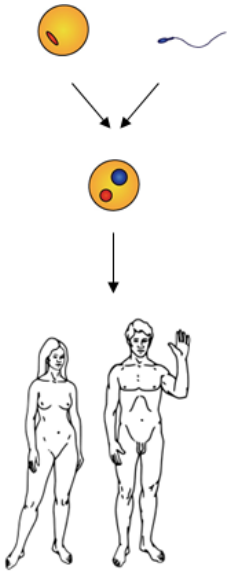
Epigenomes.

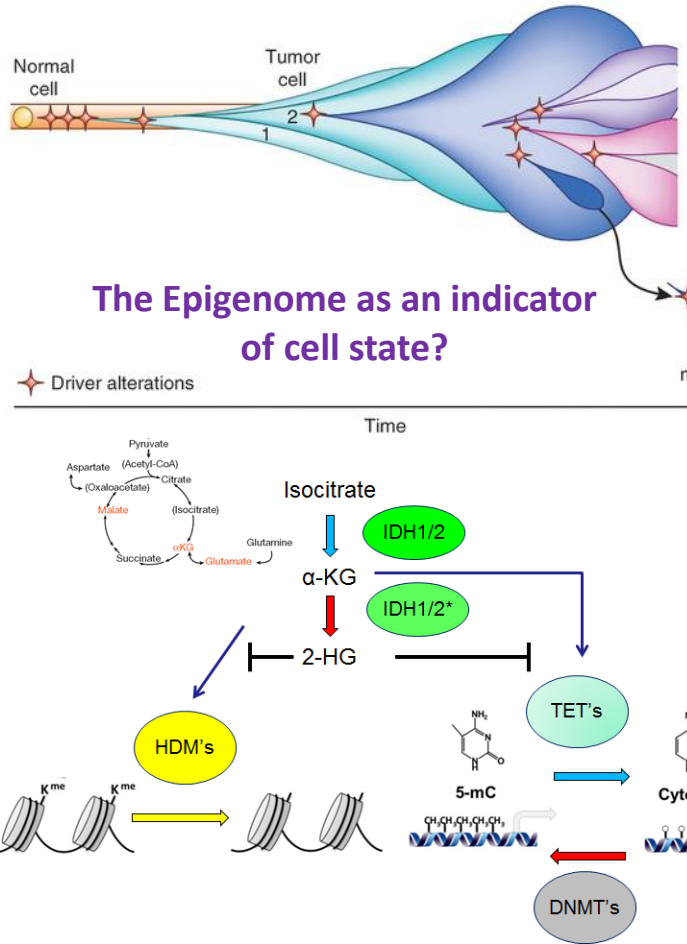


- **One genome, multiple epigenomes**

- **Epigenetics - change in gene activity in the absence of a change in DNA sequence.** The sum of mechanisms that functionally organize the genome and define cell identity: **cell state identifiers**.







REVIEW

doi:10.1038/nature09792

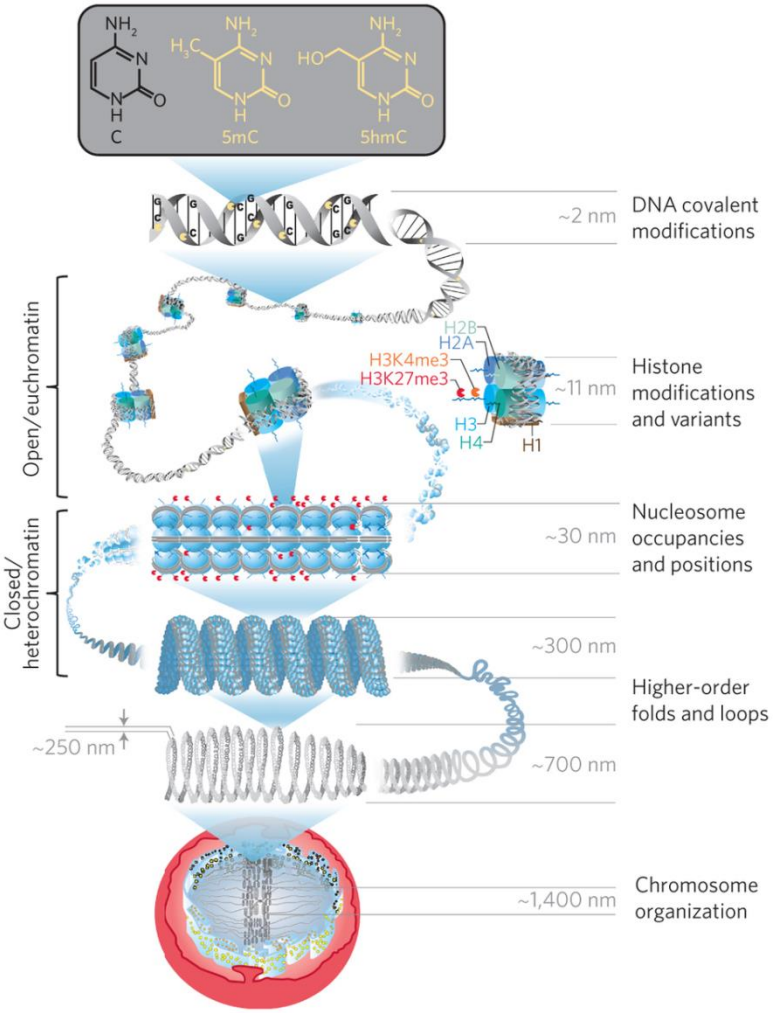
Initial impact of the sequencing of the human genome

Eric S. Lander[1]

Nature 470, 187–197 (10 February 2011)

'Ultimately, hundreds of thousands of epigenomic marks will be layered atop the genome sequence to provide an exquisite description of genomic physiology in a cell type.'

'Epigenomes and transcriptomes of humans and other species, as well as using sequencing as a proxy to probe diverse molecular interactions.'



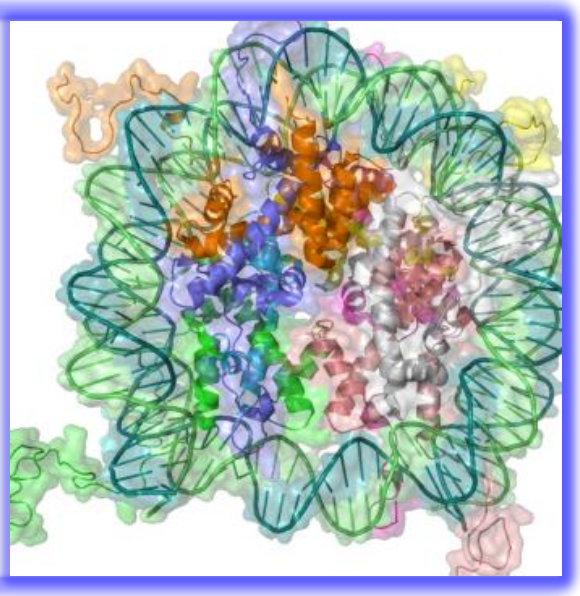
COSMIC
Catalogue of somatic mutations in cancer

http://cancer.sanger.ac.uk/cosmic

the genomic landscape of cancer

Cumulative epigenetic identity is a read out of :

- Transcriptional state
- Developmental history
- Differentiation state
- Environmental exposure
- Health and Perhaps Age

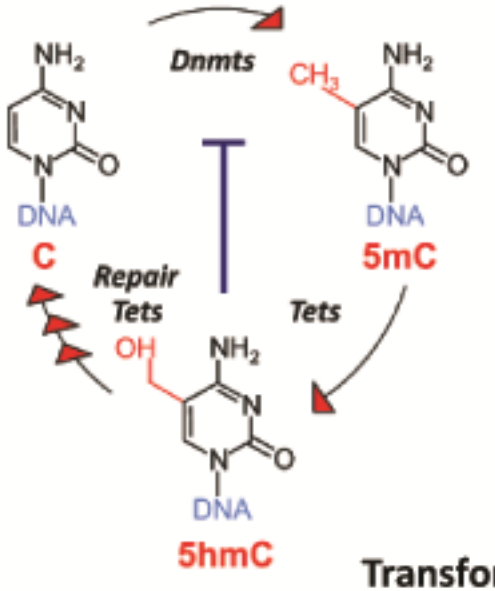


We would like a simple read-out.

To investigate Environmental exposure correlate with dose, compound etc.

Use to dissect mechanism; key for understanding risk.

DNA demethylation cycle



Development

Gene expression

Heterochromatin

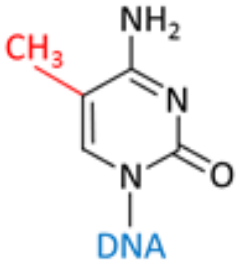
Histone modification landscapes

Higher Order Chromatin interactions

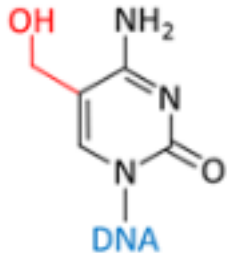
Cell Identity

Reprogramming

Transformation



5-methyl-cytosine (5mC)

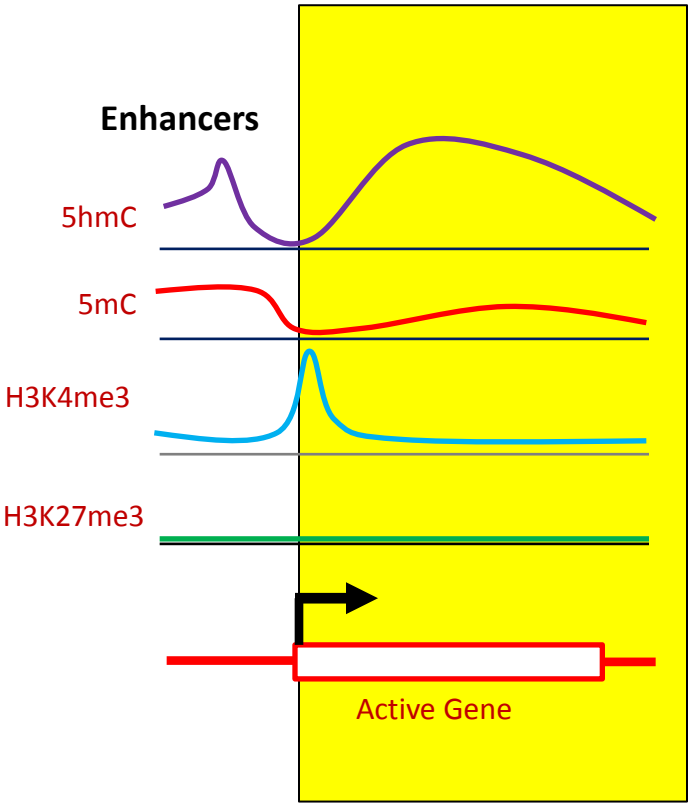


5-hydroxymethyl-cytosine (5hmC)

5-hmC a stable mark in the genome: enriched at expressed gene bodies

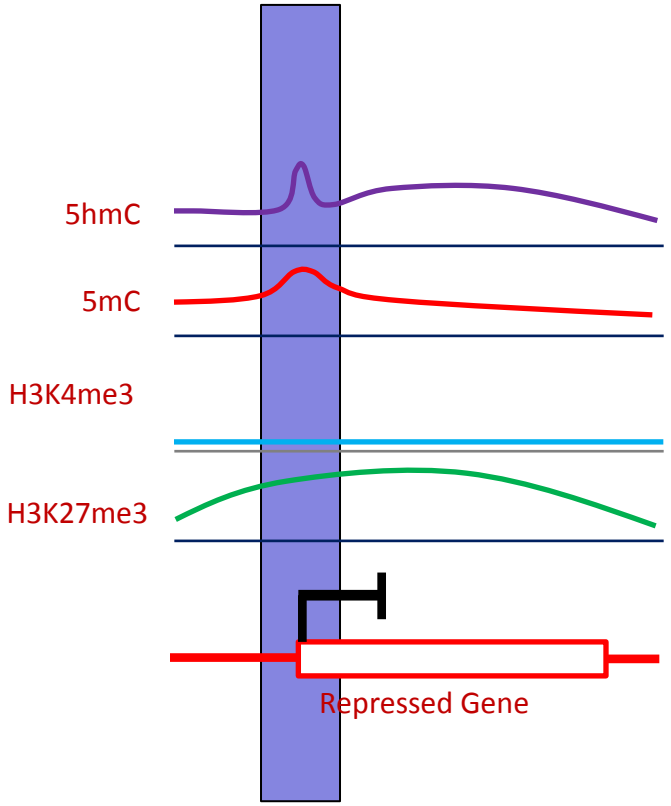
- Overall chromatin (and thus transcriptional) state is reflected through local epigenetic modifications
- Enables utilisation of epigenetic marks as indicators of 'cellular state'
- DNA modifications : 5mC (stable) and 5hmC (rapid)
- Intersect with histone tail modifications: H3K4me1, H3K27ac and H3K27me3
- Intersect with expression profiles

Gene body (Expression)



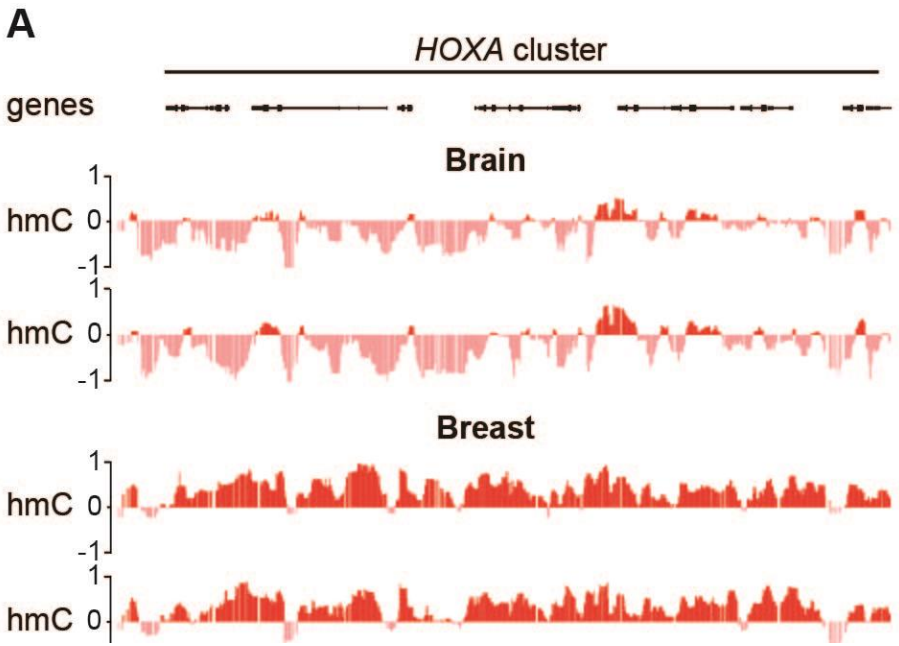
Thousands of loci

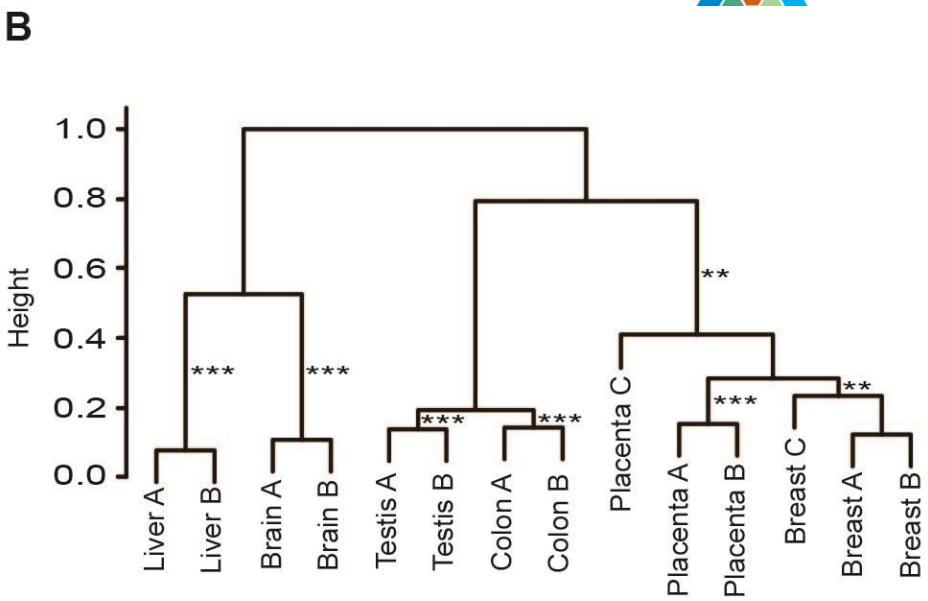
Promoter- remodelling



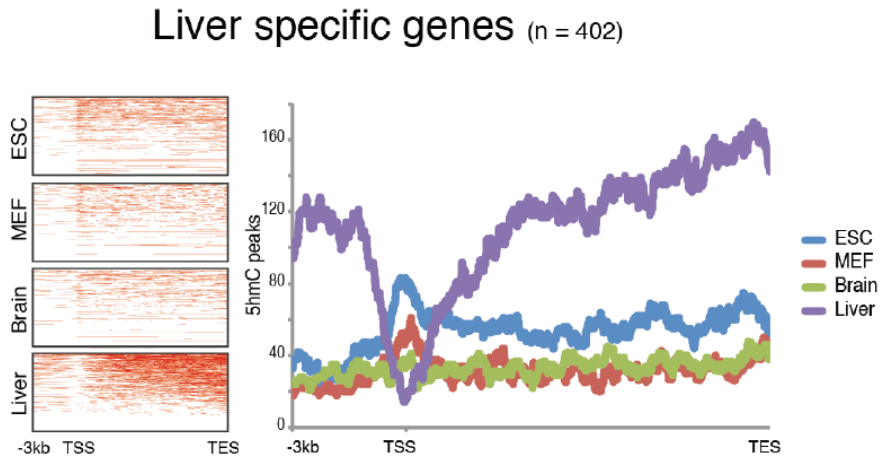
Hundreds of loci

5hmC profiles also tell you about lineage





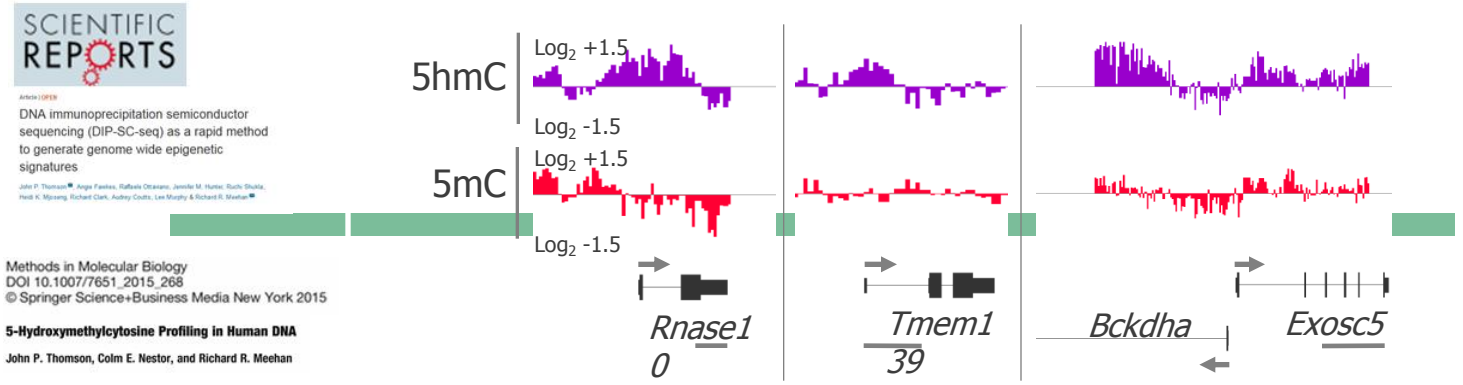
Tissue type is a major modifier of the 5-hydroxymethylcytosine content of human genes. Nestor CE, Ottaviano R, Reddington J, Sproul D, Reinhardt D, Dunican D, Katz E, Dixon JM, Harrison DJ, Meehan RR. Genome Res. 2011 Dec 19.



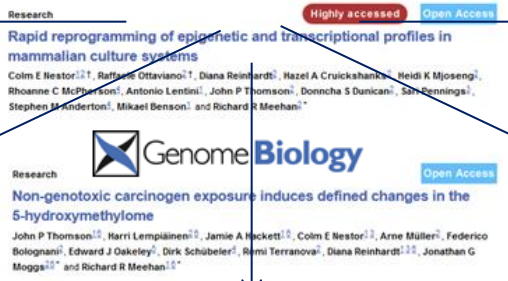
Heatmaps and distribution profiles of 5hmC in promoters and gene bodies of genes separated into two groups according to their expression level, as expressed only in liver (liver-specific).

Neri F // Oliviero S. Genome-wide analysis identifies a functional association of Tet1 and Polycomb repressive complex 2 in mouse embryonic stem cells. Genome Biol. 2013 Aug 29;14(8):R91.

Answers from the epigenetic datasets?

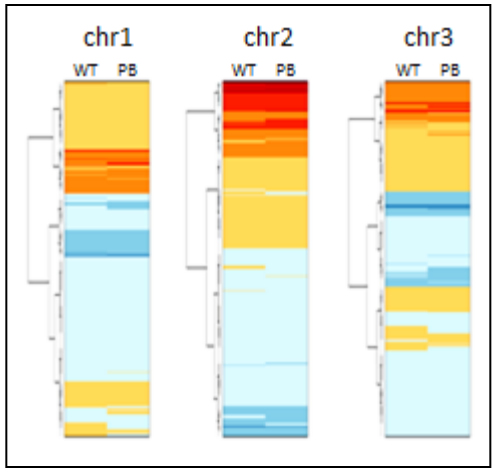


Relate to transcriptome

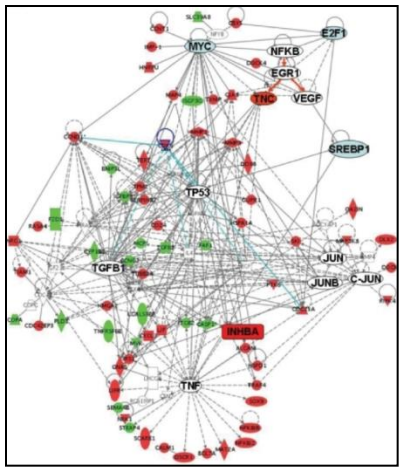


Relate to sequence of animal

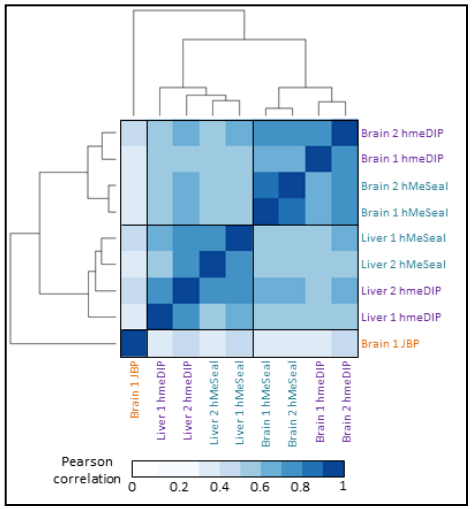
Use as "IDs"



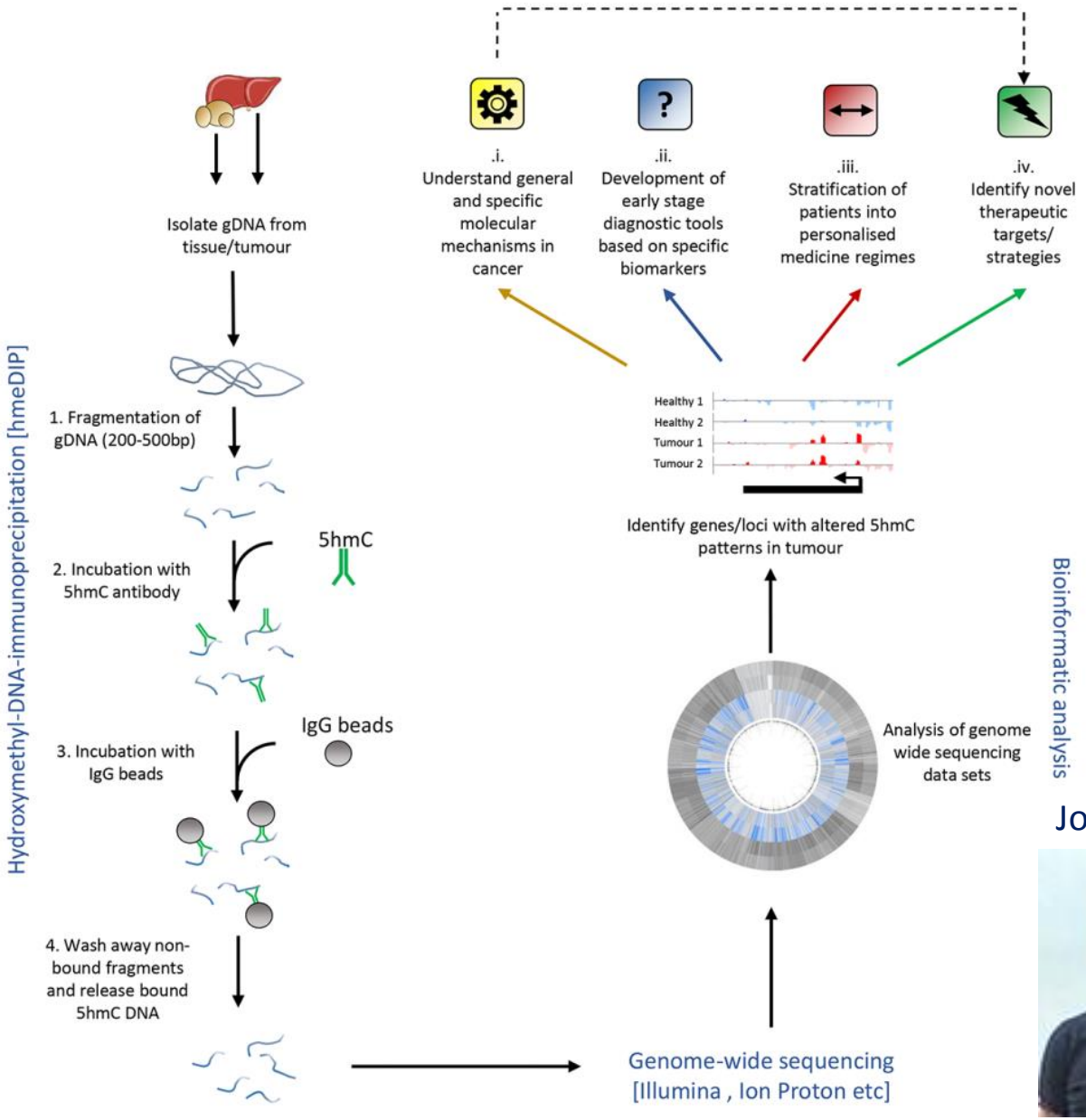
Functional GO term/pathway analysis



Correlate replicates



Study of changes to the epigenome in response to exposure to Non Genotoxic Carcinogens



John Thomson

Epigenetics in toxicity testing: a cell state indicator

- **Transcriptomic and epigenetic landscapes can be rapidly perturbed in many diseases (such as cancer) and change in response to chemical exposure**

- **Many studies now focus on combined epigenomic and transcriptomic analysis in toxicity testing.**

- **One aim of these studies is to define characteristic early stage biomarkers, which may impact on down-stream events such as tumour formation.**

- **Reduce the three Rs in toxicity testing**
  - Replacement (of animal models with cell culture models)
  - Reduction (in numbers required epigenetic vs genetics)
  - Refinement (reduced length of study time vs long term phenotypic studies)

John Thomson

Justification:

What is the 5mC and 5hmC ground state of commonly used rodent (rat and mouse) strains used in Toxicity testing?

Focus:

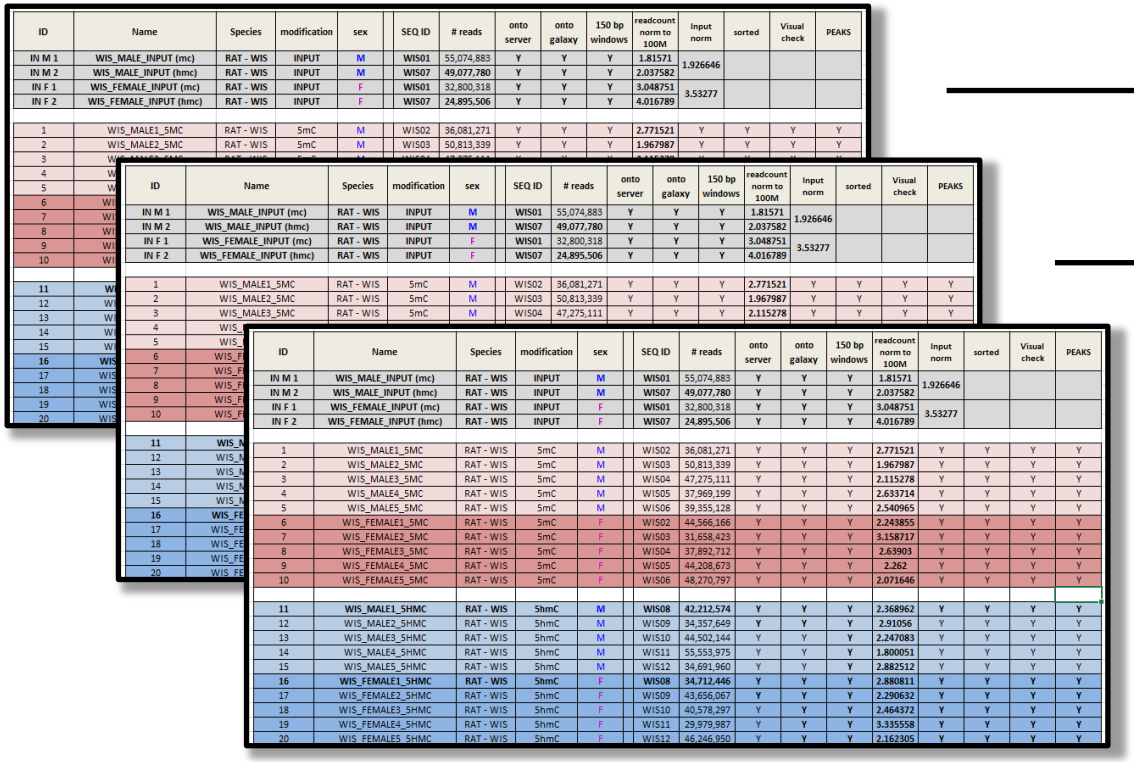
Liver tissue

Strains:

5x male and 5x female Sprague Dawley
5x male and 5x female Wistar Dawley

5x male and 5x female CD1 outbred mouse strain

We carried out 60x (5 male, 5 female for SD, WIS and CD1 for 5mC , same for 5hmC) DNA extractions , immunoprecipitations, amplifications, library preparations and Genome wide sequencing runs.



ID	Name	Species	modification	sex	SEQ ID	# reads	onto server	onto galaxy	150 bp windows	readcount norm to 100M	Input norm	sorted	Visual check	PEAKS
IN M 1	WIS_MALE_INPUT (mc)	RAT - WIS	INPUT	M	WIS01	55,074,883	Y	Y	Y	1.81571	1.926646			
IN M 2	WIS_MALE_INPUT (hmc)	RAT - WIS	INPUT	M	WIS07	49,077,780	Y	Y	Y	2.037582				
IN F 1	WIS_FEMALE_INPUT (mc)	RAT - WIS	INPUT	F	WIS01	32,800,318	Y	Y	Y	3.048751	3.53277			
IN F 2	WIS_FEMALE_INPUT (hmc)	RAT - WIS	INPUT	F	WIS07	24,895,506	Y	Y	Y	4.016789				
1	WIS_MALE1_5MC	RAT - WIS	5mC	M	WIS02	36,081,271	Y	Y	Y	2.771521	Y	Y	Y	Y
2	WIS_MALE2_5MC	RAT - WIS	5mC	M	WIS03	50,813,339	Y	Y	Y	1.967987	Y	Y	Y	Y
3	WIS_MALE3_5MC	RAT - WIS	5mC	M	WIS04	47,275,111	Y	Y	Y	2.115278	Y	Y	Y	Y
4	WIS_MALE4_5MC	RAT - WIS	5mC	M	WIS05	37,969,199	Y	Y	Y	2.633714	Y	Y	Y	Y
5	WIS_MALE5_5MC	RAT - WIS	5mC	M	WIS06	39,355,128	Y	Y	Y	2.540965	Y	Y	Y	Y
6	WIS_FEMALE1_5MC	RAT - WIS	5mC	F	WIS02	44,566,166	Y	Y	Y	2.243855	Y	Y	Y	Y
7	WIS_FEMALE2_5MC	RAT - WIS	5mC	F	WIS03	31,658,423	Y	Y	Y	3.158717	Y	Y	Y	Y
8	WIS_FEMALE3_5MC	RAT - WIS	5mC	F	WIS04	37,892,712	Y	Y	Y	2.63903	Y	Y	Y	Y
9	WIS_FEMALE4_5MC	RAT - WIS	5mC	F	WIS05	44,208,673	Y	Y	Y	2.262	Y	Y	Y	Y
10	WIS_FEMALE5_5MC	RAT - WIS	5mC	F	WIS06	48,270,797	Y	Y	Y	2.071646	Y	Y	Y	Y
11	WIS_MALE1_5HMC	RAT - WIS	5hmC	M	WIS08	42,212,574	Y	Y	Y	2.368962	Y	Y	Y	Y
12	WIS_MALE2_5HMC	RAT - WIS	5hmC	M	WIS09	34,357,649	Y	Y	Y	2.91056	Y	Y	Y	Y
13	WIS_MALE3_5HMC	RAT - WIS	5hmC	M	WIS10	44,502,144	Y	Y	Y	2.247083	Y	Y	Y	Y
14	WIS_MALE4_5HMC	RAT - WIS	5hmC	M	WIS11	55,553,975	Y	Y	Y	1.800051	Y	Y	Y	Y
15	WIS_MALE5_5HMC	RAT - WIS	5hmC	M	WIS12	34,691,960	Y	Y	Y	2.882512	Y	Y	Y	Y
16	WIS_FEMALE1_5HMC	RAT - WIS	5hmC	F	WIS08	34,712,446	Y	Y	Y	2.880811	Y	Y	Y	Y
17	WIS_FEMALE2_5HMC	RAT - WIS	5hmC	F	WIS09	43,656,067	Y	Y	Y	2.290632	Y	Y	Y	Y
18	WIS_FEMALE3_5HMC	RAT - WIS	5hmC	F	WIS10	40,578,297	Y	Y	Y	2.464372	Y	Y	Y	Y
19	WIS_FEMALE4_5HMC	RAT - WIS	5hmC	F	WIS11	29,979,987	Y	Y	Y	3.335558	Y	Y	Y	Y
20	WIS_FEMALE5_5HMC	RAT - WIS	5hmC	F	WIS12	46,246,950	Y	Y	Y	2.162305	Y	Y	Y	Y

Also profiling H3K27ac and H3K4me3- enhancer and expression marks

Mapping, processing of data and analysis of 60 x DNA modification datasets (each >20 million rows in length)

2.4 Billion raw reads converted into **1.2 Billion** rows of DNA modification data

DNA mod seq run 20/02/2015- 1/2/2016 (347 days)

All data was internally normalised to input sequences in order to infer true enrichment over sequencing depth. Thus a signal > 0 = more reads in the enriched samples vs background noise.

Conclusions to date

- Have successfully generated the first comprehensive DNA modification maps (5hmC and 5mC) for two strains of rat liver and one strain of mouse liver

- Global 5hmC and 5mC patterns show **that variance within a strain and gender is low** (good Pearson's correlation values)

- Epigenetic patterns reflect genetic makeup: strain cluster before genders

- The majority of variance is found within genes but analysis of genic levels alone does not stratify genders/strains (genic = more conserved regions of genome). Thus lots of low levels intragenic variance likely to be responsible for the global clustering.

- Small numbers of genes routinely variable between strains (i.e. WIS specific enrichment at given locus)

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