

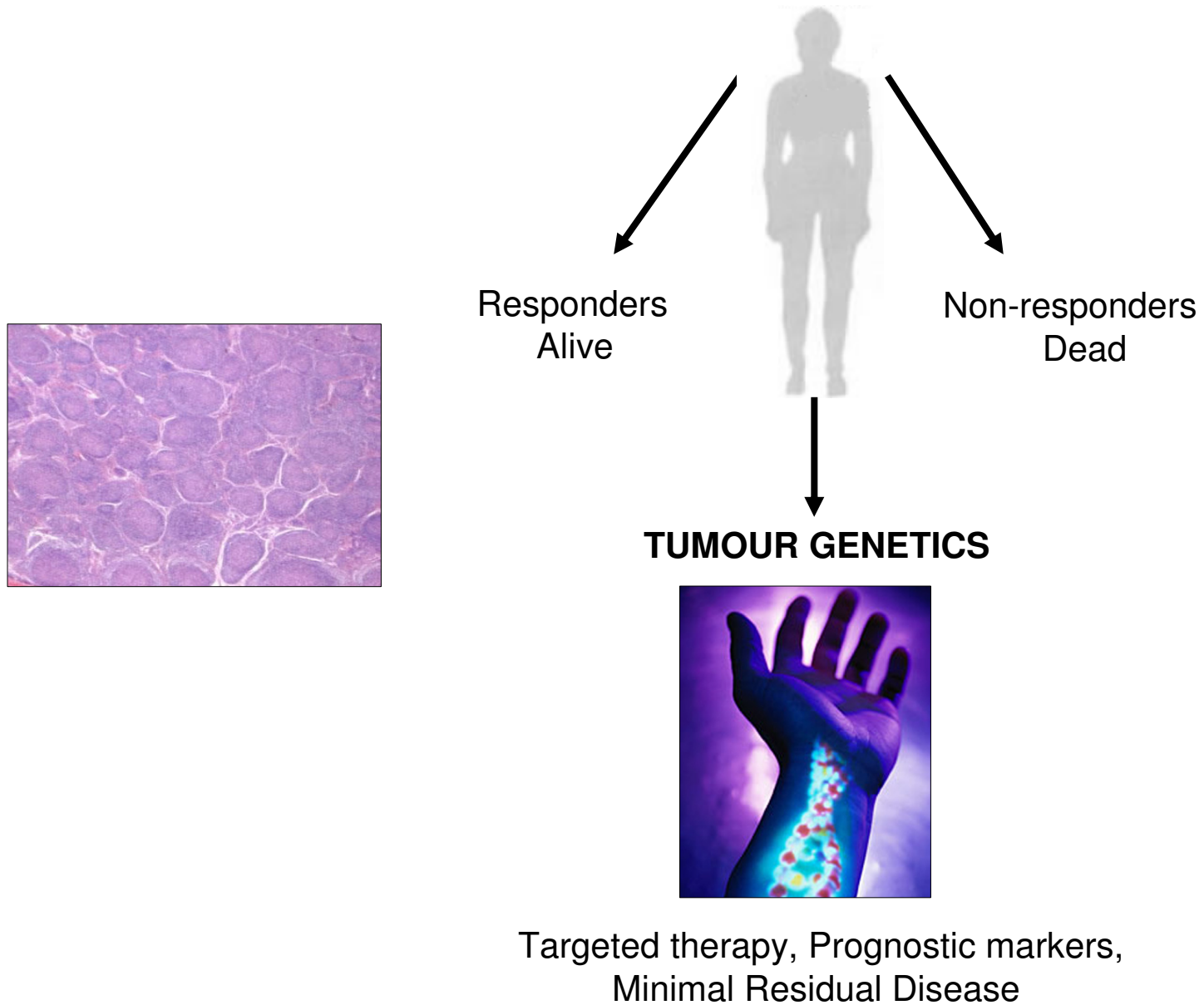


FOLLICULAR LYMPHOMA- ILLUMINA METHYLATION

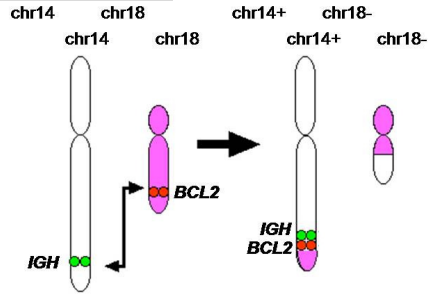
Jude Fitzgibbon

j.fitzgibbon@qmul.ac.uk

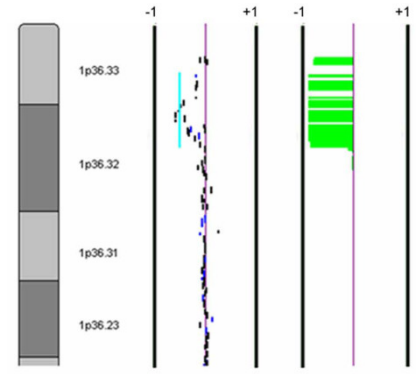
Molecular Predictors of Clinical outcome



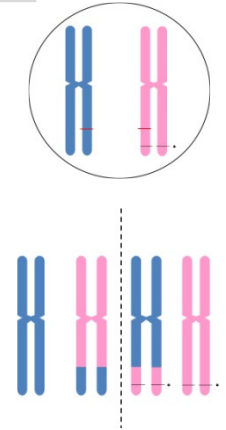
t(14;18)



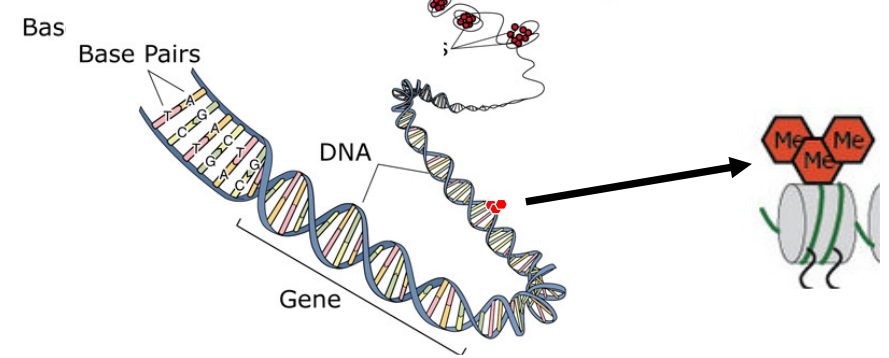
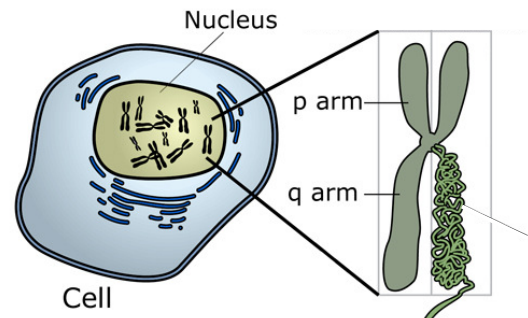
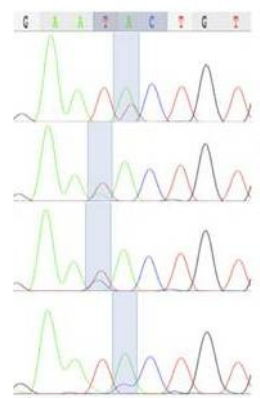
CN changes



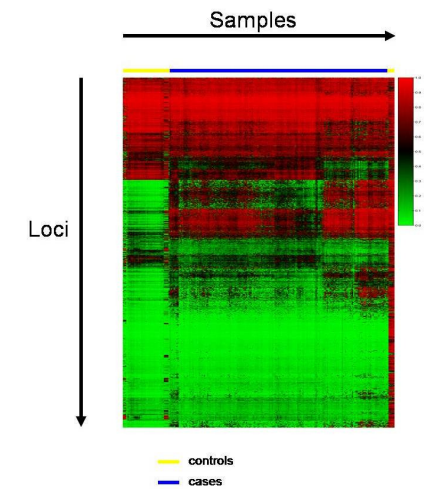
UPD



Mutation

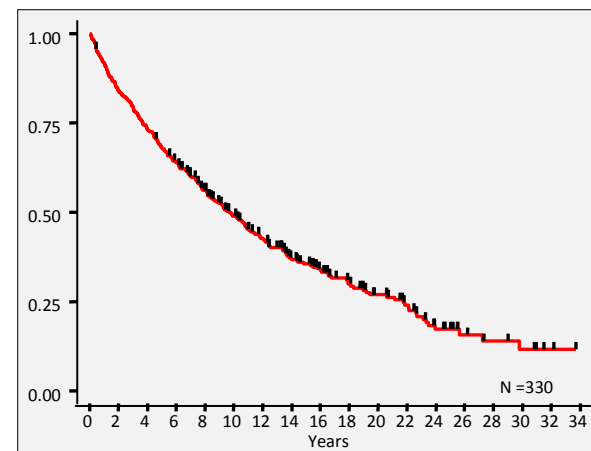
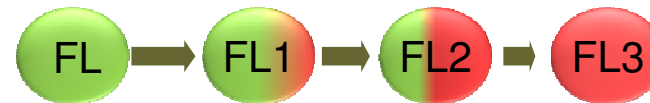
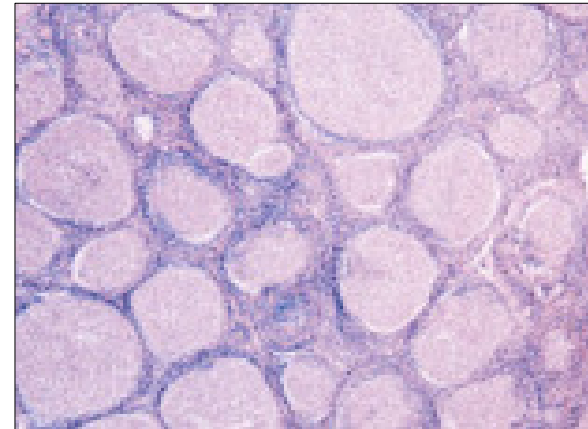


Methylation

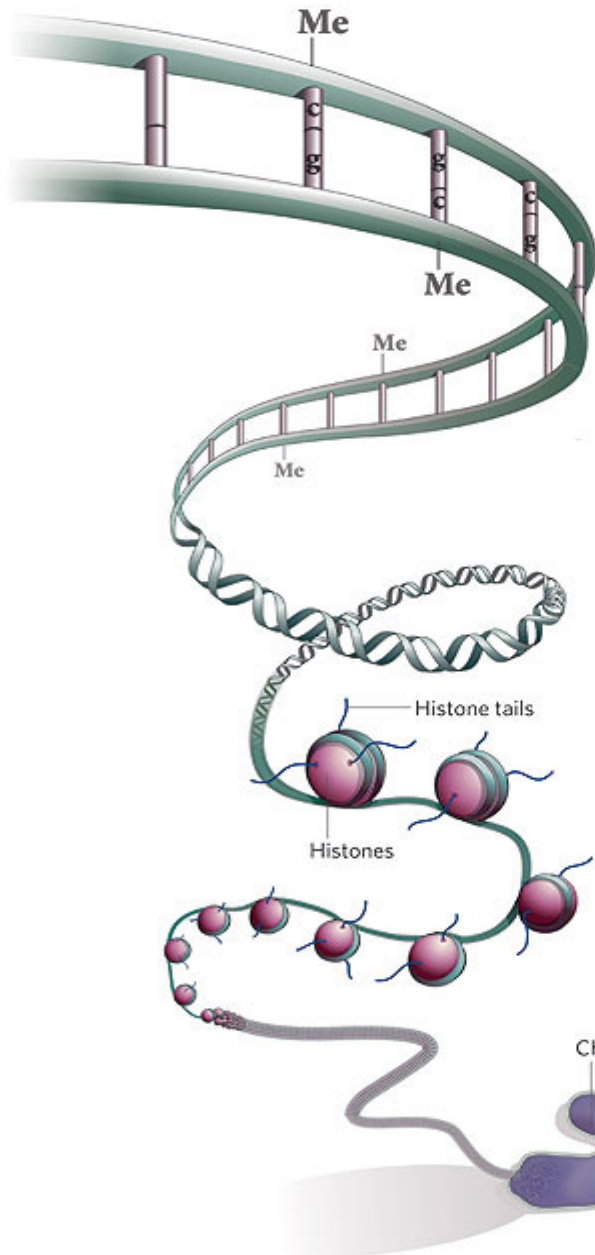


Features of Follicular Lymphoma

- GC B-cell disease
- $t(14;18) \gg$ IgH-Bcl2
- 2000 new cases in UK
- 10 yr median survival
- Relapse-remitting
- Rituximab
- Transformation: 1-2yr survival
- Epigenetics



Epigenetic Modifications



The two main components
of the epigenetic code

**DNA
Methylation**

**Histone
Modifications**



Regulation of gene expression

Published Literature in FL - Individual Genes

Malignant Lymphomas • Brief Report

DAP-kinase hypermethylation in the bone marrow of patients with follicular lymphoma

The Androgen Receptor Gene is Preferentially Hypermethylated in Follicular Non-Hodgkin's Lymphomas

Letters to the Editor

ABF-1 is frequently silenced by promoter methylation in follicular lymphoma, diffuse large B-cell lymphoma and Burkitt's lymphoma

Frequent epigenetic inactivation of *Rb1* in addition to *p15* and *p16* in mantle cell and follicular lymphoma[☆]

C.S. Chim MBChB, MD, PhD, FRCP, FACP^{a,}, K.Y. Wong BSc^b, F. Loong, MBBS, FRCPA^b, W.W. Lam MBBS, FRCPA^c, G. Srivastava PhD^{b,*}*

Aberrant DNA methylation of *p57^{KIP2}* gene in the promoter region in lymphoid malignancies of B-cell phenotype

Yinghua Li, Hirokazu Nagai, Toshihito Ohno, Masaaki Yuge, Sonoko Hatano, Etsuro Ito, Naoyoshi Mori, Hidehiko Saito, and Tomohiro Kinoshita

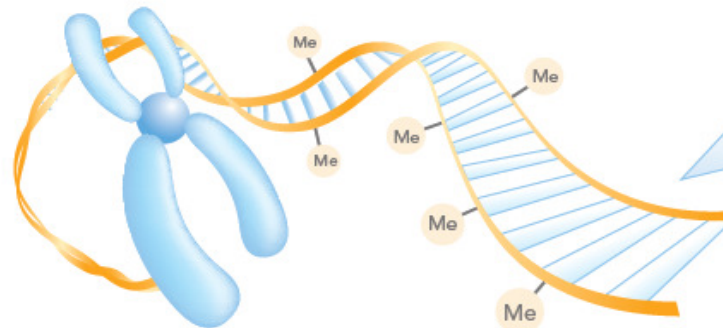
Array-based Methylation Analysis (Illumina®)

GoldenGate - 1500.....

Infinium HumanMethylation27- BeadChip 27000.....

New Platform >400,000

£50.....£220.....C£220



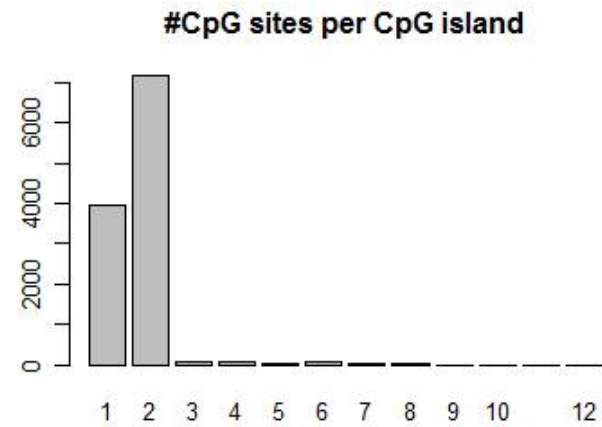
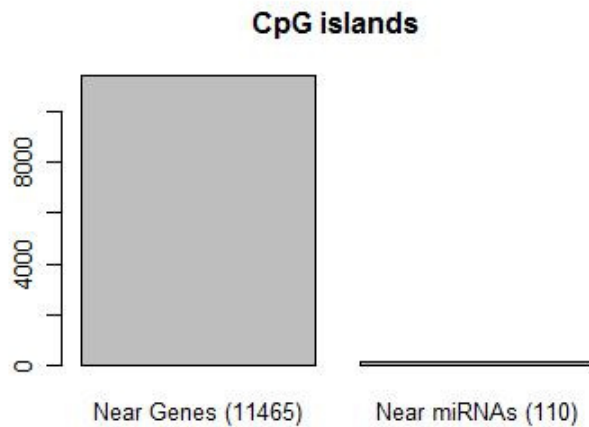
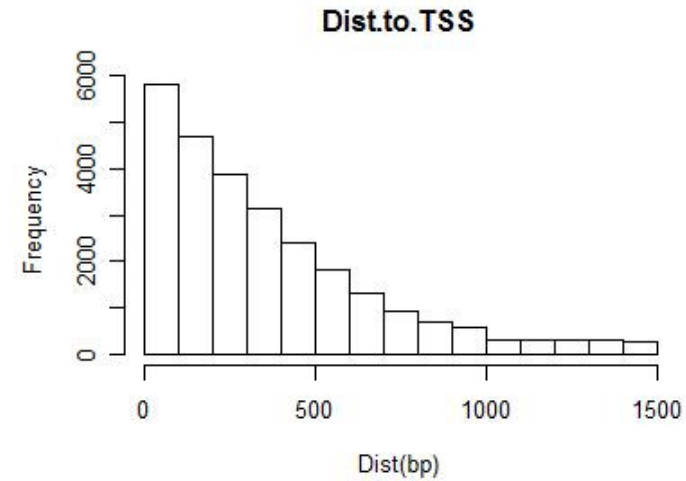
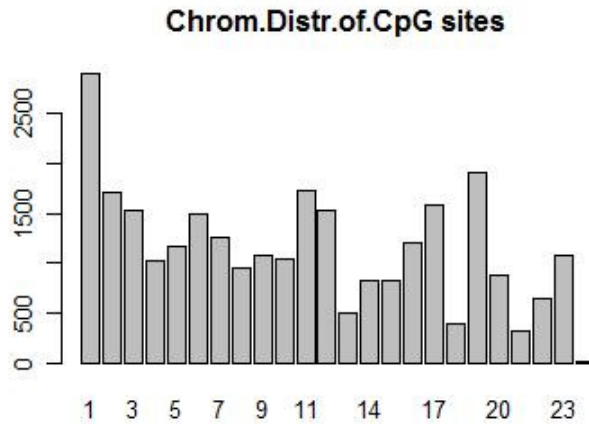
Infinium HumanMethylation27 BeadChip

Quantitative methylation measurement at the single-CpG level

- 27 578 CpG loci
- 14 000 genes
- 1000 cancer related genes
- 200 microRNA promoters
- 12 sample BeadArray format



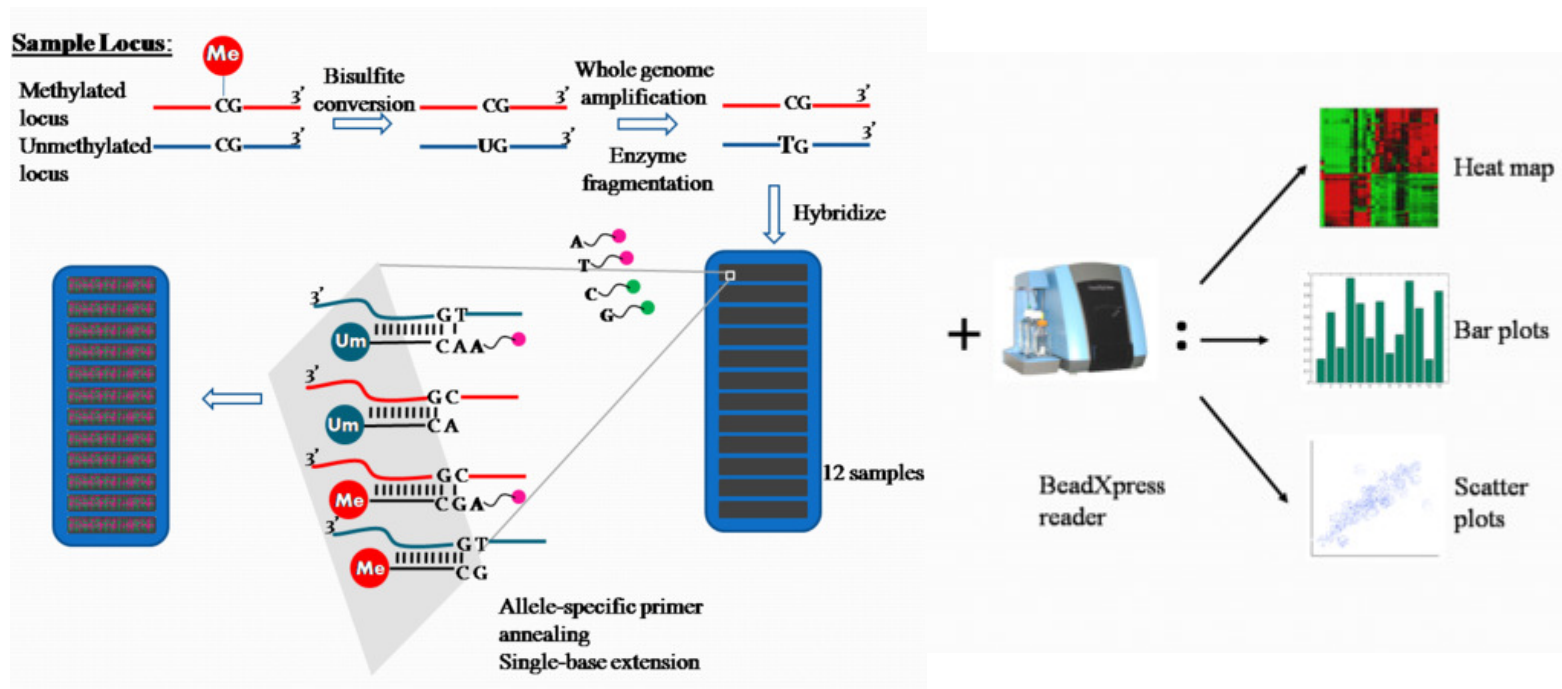
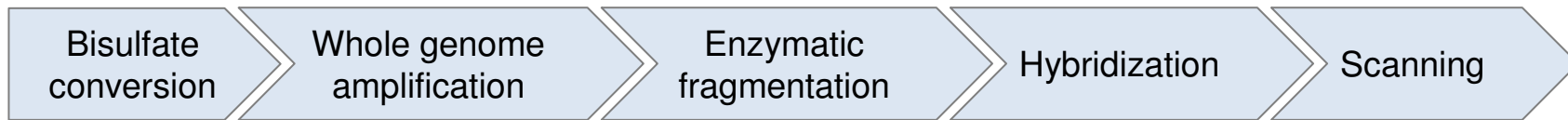
Infinium HumanMethylation27 BeadChip



Courtesy of Dr Andrew Teschendorff- UCL

Infinium HumanMethylation27 BeadChip

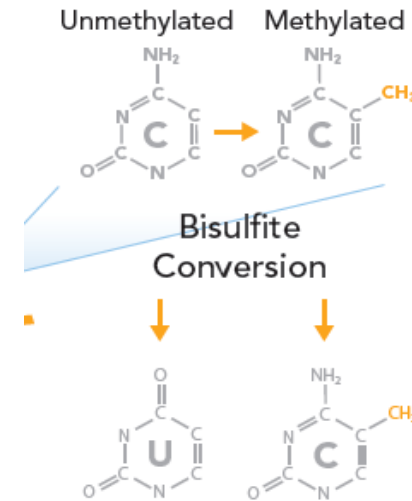
The workflow ...



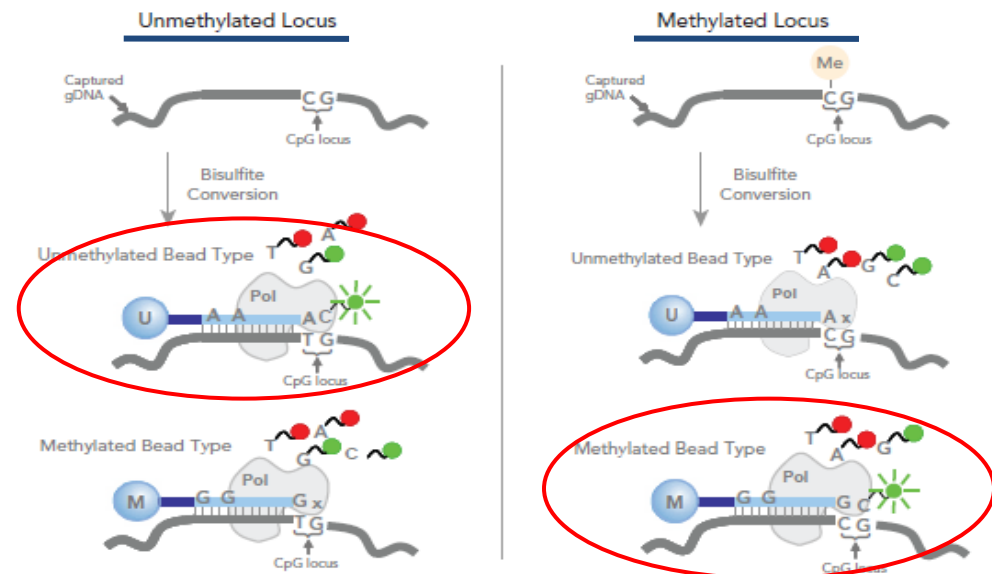
Infinium HumanMethylation27 BeadChip

Background

- Bisulfate modification:
 - unmethylated C converted to U
 - methylated C protected from conversion
- Site specific probes
 - U & M bead type (unmethylated and methylated)
 - hybridization followed by a single base extension

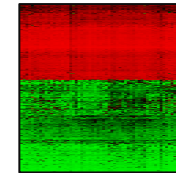
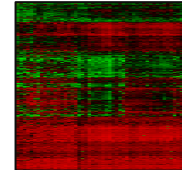


- Continuous measurement:
β value: 0-1



Aim of the Project

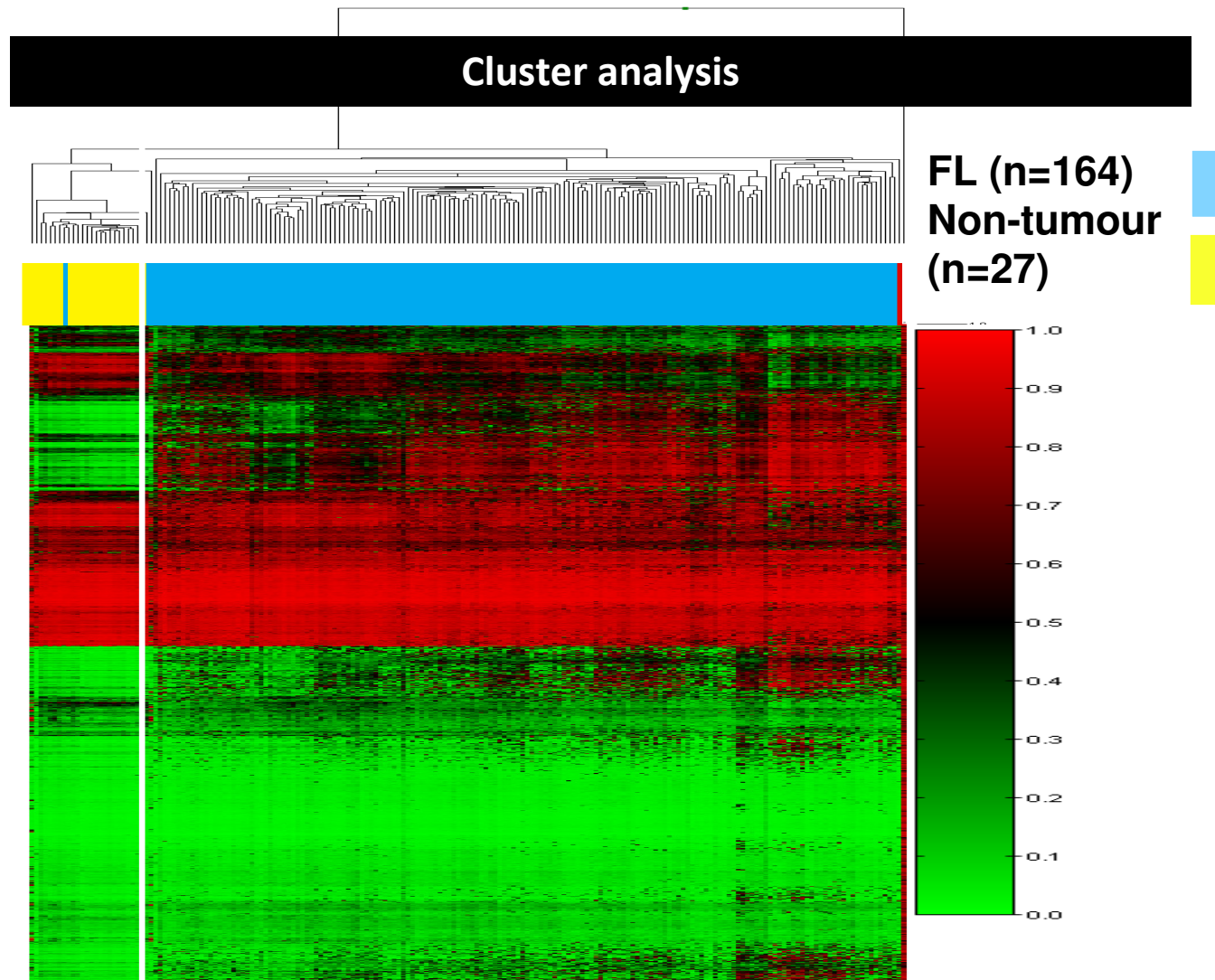
- Determine extent and frequency of methylation in FL
- Correlate with gene expression
- Correlate with clinical data
- Follow anything exciting



Methylation Profiling – Samples

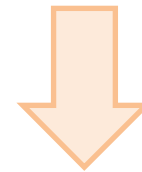
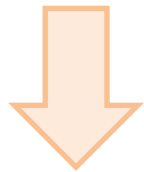
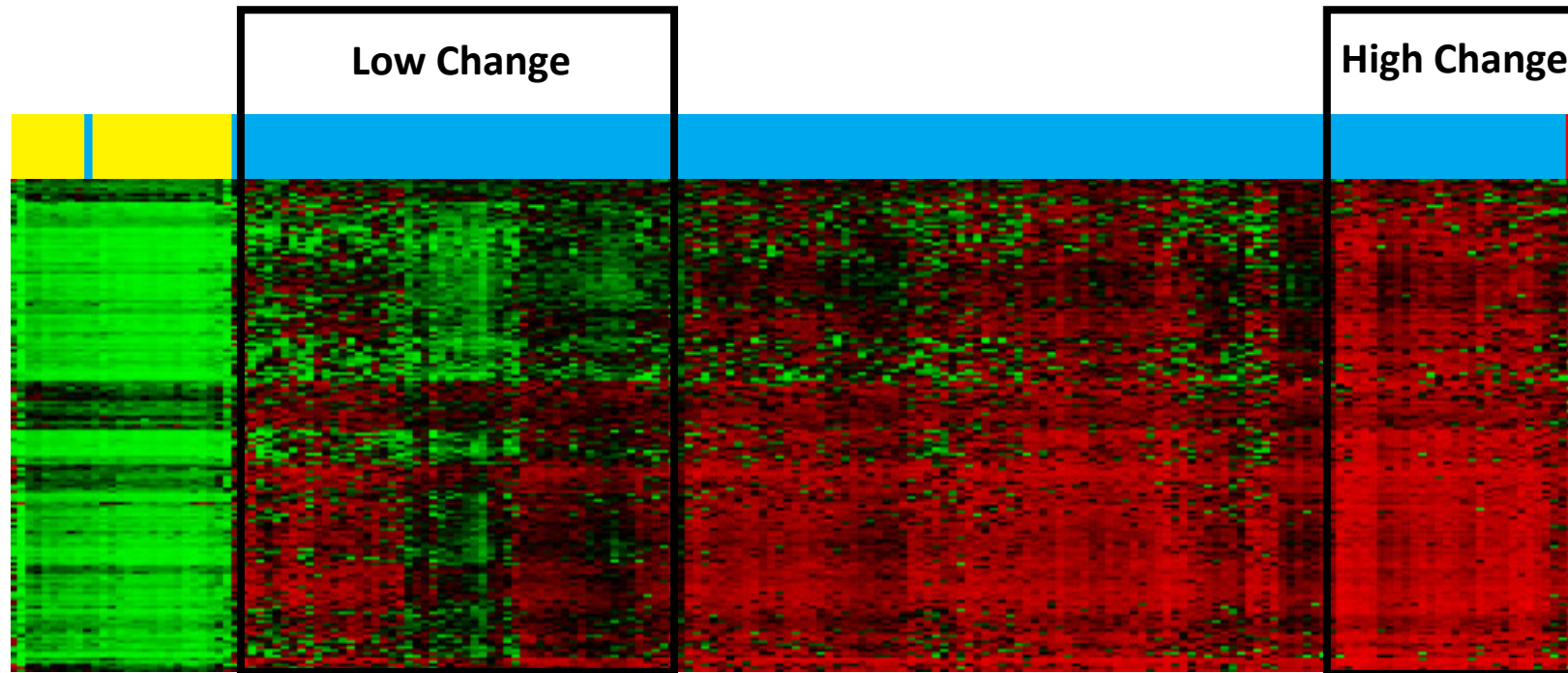
Sample	n	Mean Age, (Range)
Malignant Lymph Node DNA	184	
Previously untreated Follicular Lymphoma (FL)	164	52 (23-90)
Paired FL and transformed FL:		
Pre-transformation FL	10	
Transformed FL	10	
Benign Lymph Node DNA*	19	27 (4-55)
Hyperplastic	14	
Granulomatous	3	
PTGC	3	
Dermatopathic lymphadenitis	2	
Granulation Tissue	1	
Tonsils / Adenoids	4	13 (7-27)
Hyperplastic	4	

Methylation Profiling – Cluster analysis



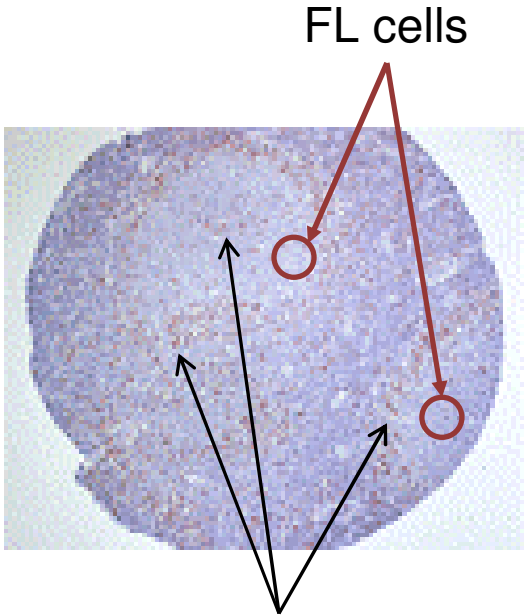
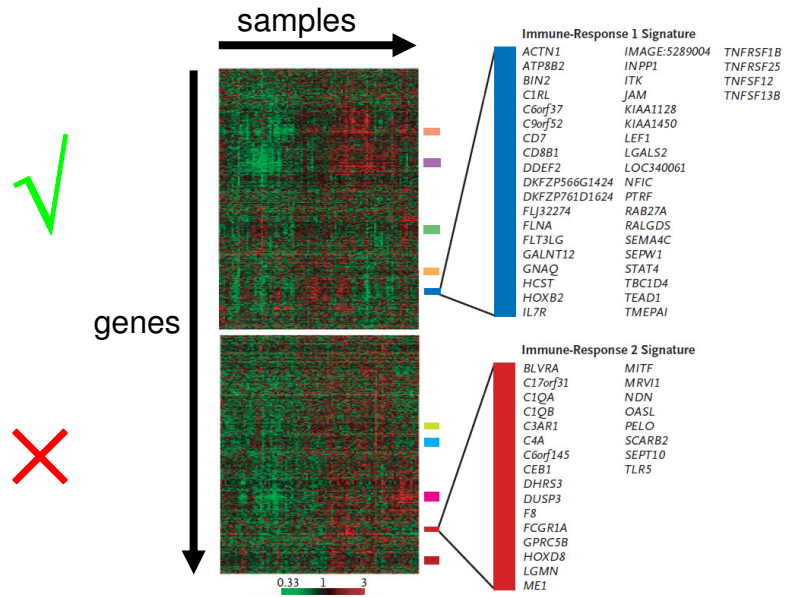
- Robust discriminator between malignant vs benign samples
O' Riain et al, Leukemia 2009

Methylation Profiling – Cluster analysis



No difference in age, grade, stage, clinical outcome between extremes !

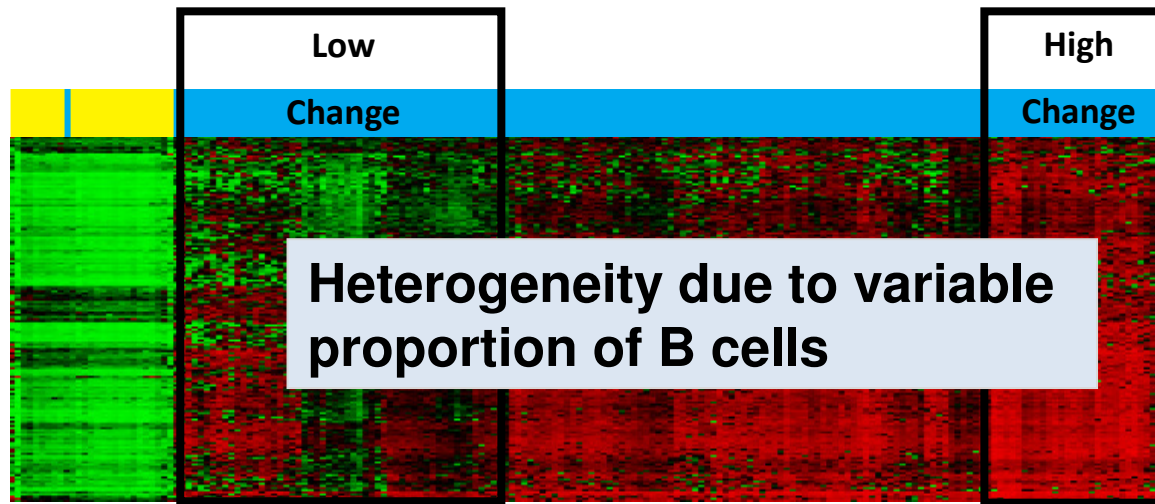
Microenvironment



Gene-Expression Signature	P Value for Contribution to Model in Test Set	Relative Risk of Death (95% CI) ^a	Effect of Increased Gene Expression on Survival
Immune-response 1	<0.001	0.15 (0.05–0.46)	Favorable
Immune-response 2	<0.001	9.35 (3.02–28.90)	Unfavorable

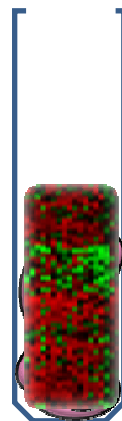
Dave et al NEJM 2004

Methylation Profiling – Cluster analysis

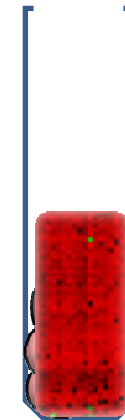


CELL SORTING:

CD3+ T cells



CD19+ B cells



The Role of Polycomb Repressor Complex 2

nature
genetics

LETTERS

2007: enrichment for PRC2 target genes among hypermethylated gene sets in carcinoma

A stem cell–like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing

Joyce E Ohm¹, Kelly M McGarvey^{1,2}, Xiaobing Yu³, Linzhao Cheng^{2–4}, Kornel E Schuebel¹, Leslie Cope⁴, Helai P Mohammad¹, Wei Chen^{1,5}, Vincent C Daniel¹, Wayne Yu¹, David M Berman⁶, Thomas Jenuwein⁷, Kevin Pruitt¹, Saul J Sharkis^{1,2}, D Neil Watkins¹, James G Herman^{1,2} & Stephen B Baylin^{1,2}

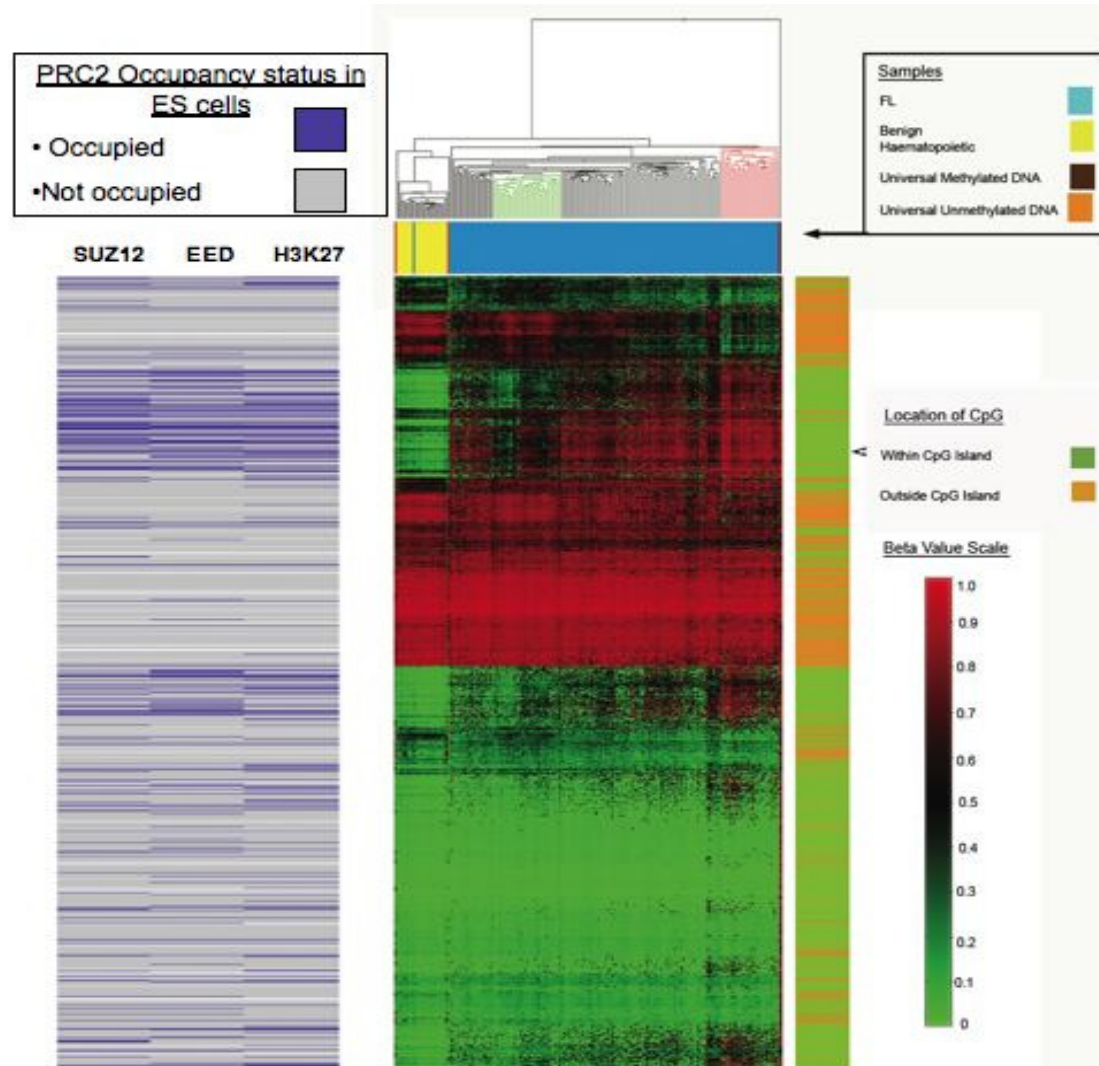
Epigenetic stem cell signature in cancer

Martin Widschwendter¹, Heidi Fieg^{1,2}, Daniel Egle², Elisabeth Mueller-Holzner², Gilbert Spizzo³, Christian Marth², Daniel J Weisenberger⁴, Mihaela Campan⁴, Joanne Young⁵, Ian Jacobs¹ & Peter W Laird⁴

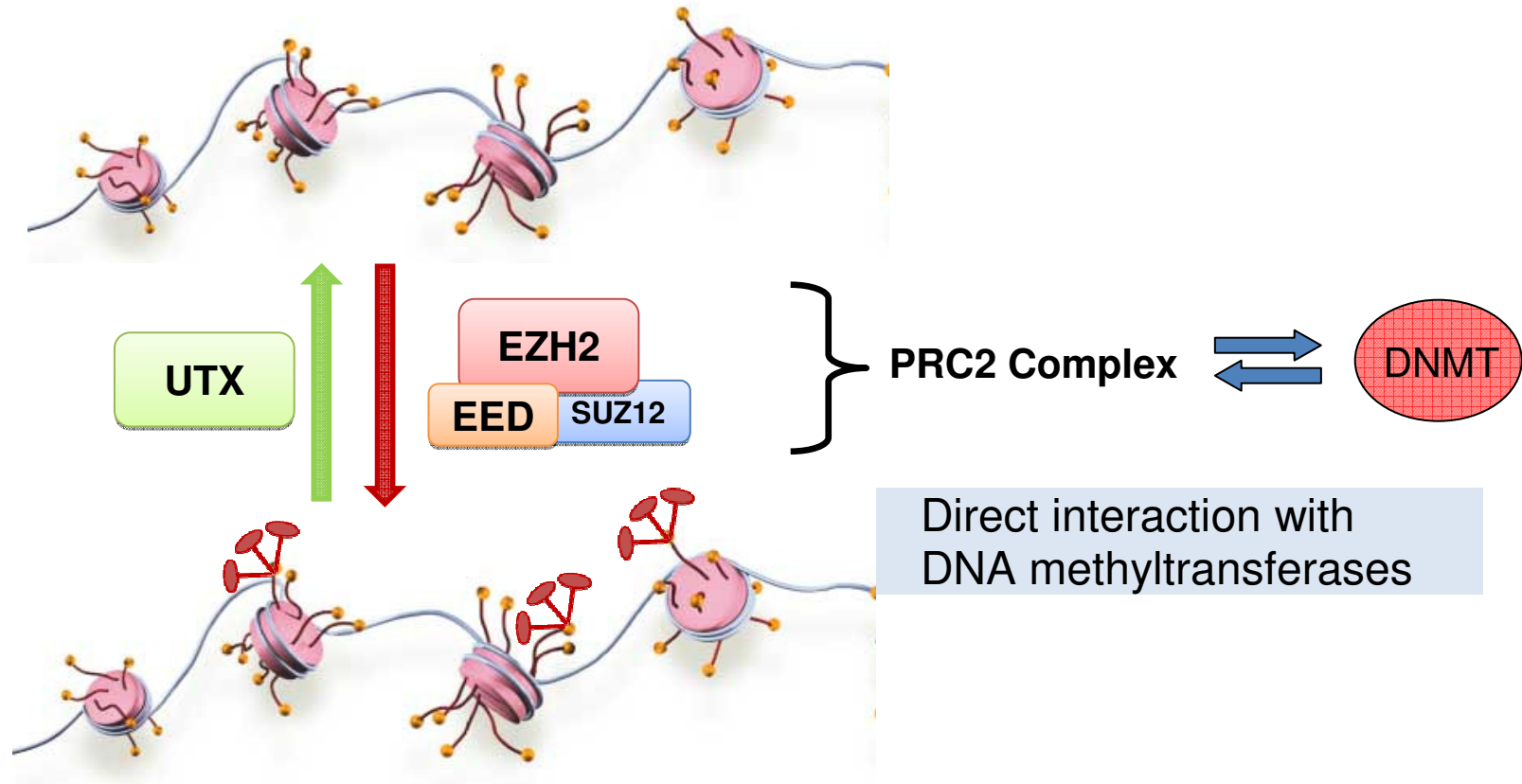
Polycomb-mediated methylation on Lys27 of histone H₃ pre-marks genes for *de novo* methylation in cancer

Yeshayahu Schlesinger¹, Ravid Straussman¹, Ilana Keshet¹, Shlomit Farkash², Merav Hecht¹, Joseph Zimmerman³, Eran Eden⁴, Zohar Yakhini^{4,5}, Etti Ben-Shushan⁶, Benjamin E Reubinoff⁶, Yehudit Bergman⁷, Itamar Simon² & Howard Cedar¹

Hypermethylated genes -PRC2 targets in ES cells.

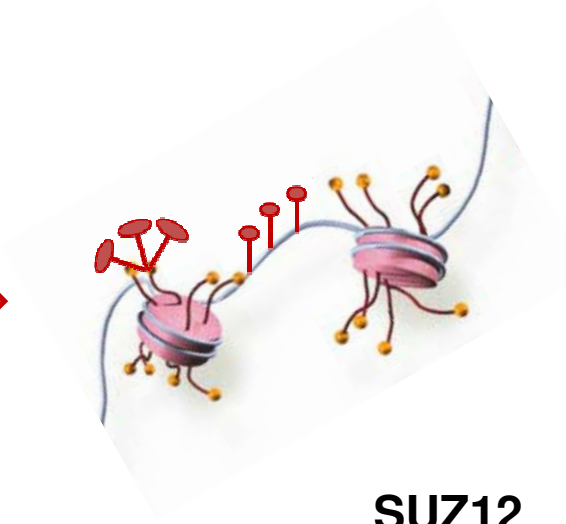
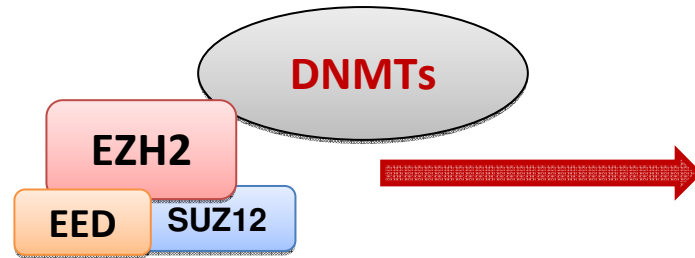


The PRC2 complex and Histone methylation



***Trimethylated H3K27
Repressive chromatin mark***

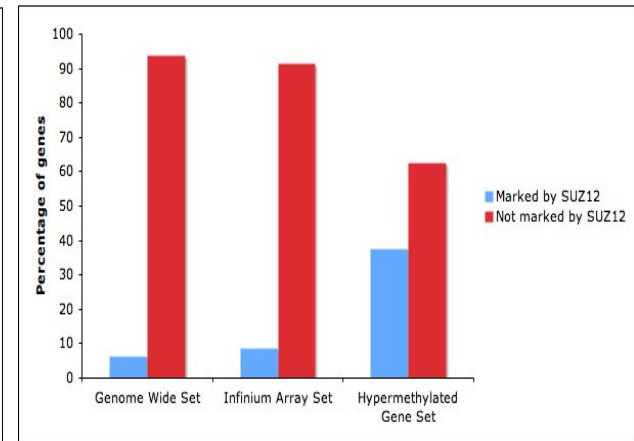
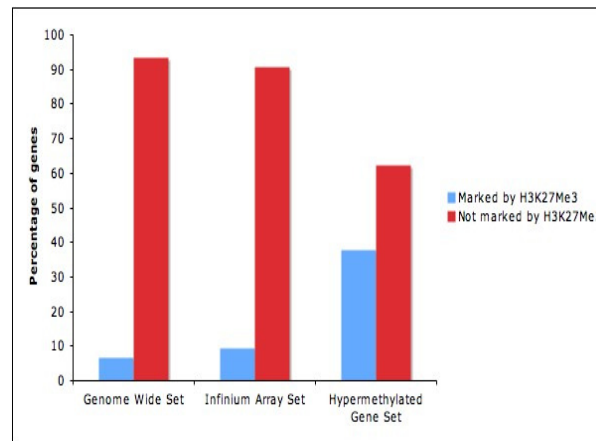
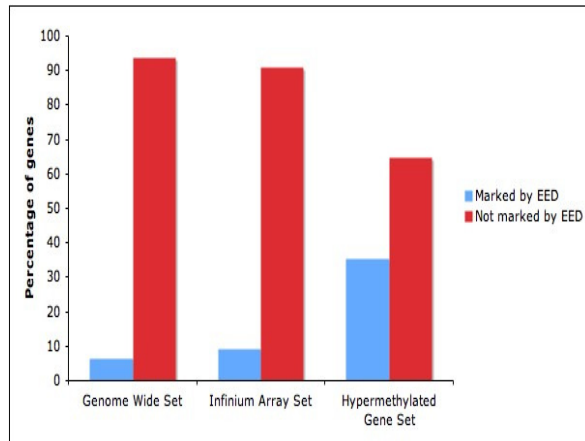
Link PRC2 and Hypermethylation loci in FL



EED

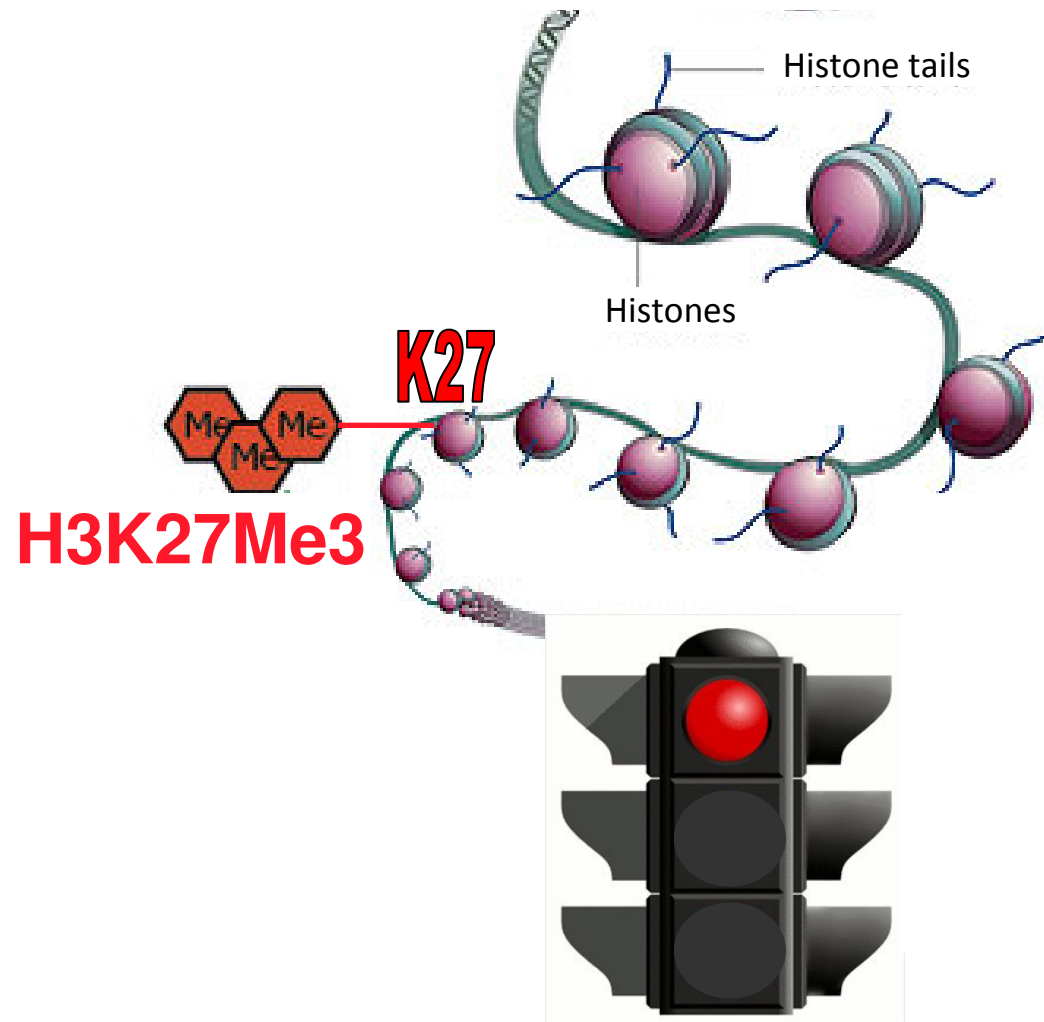
EZH2

SUZ12



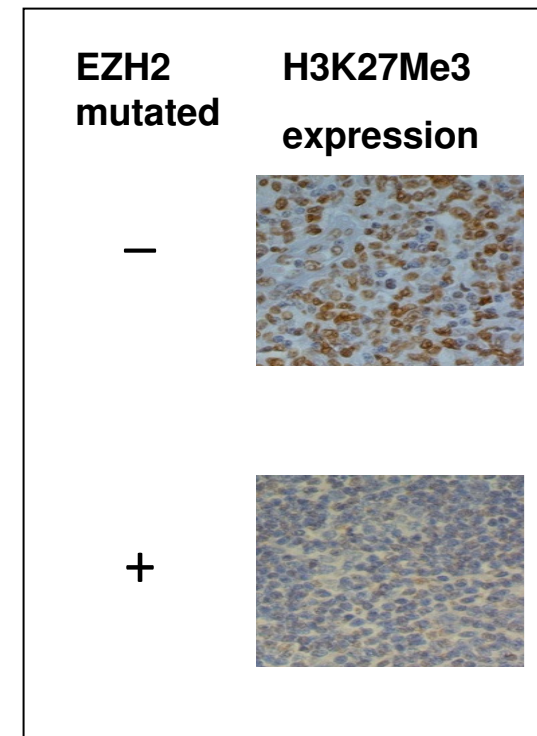
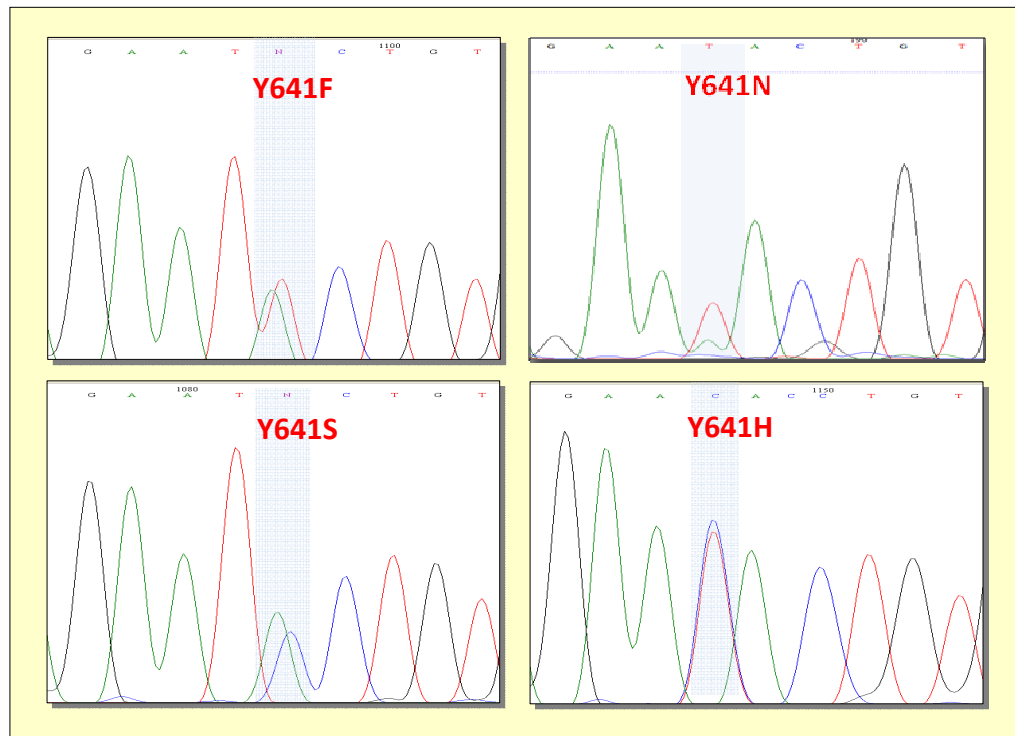
Histone Methyl marks (K4, K27)

- Gene expression **stopped**



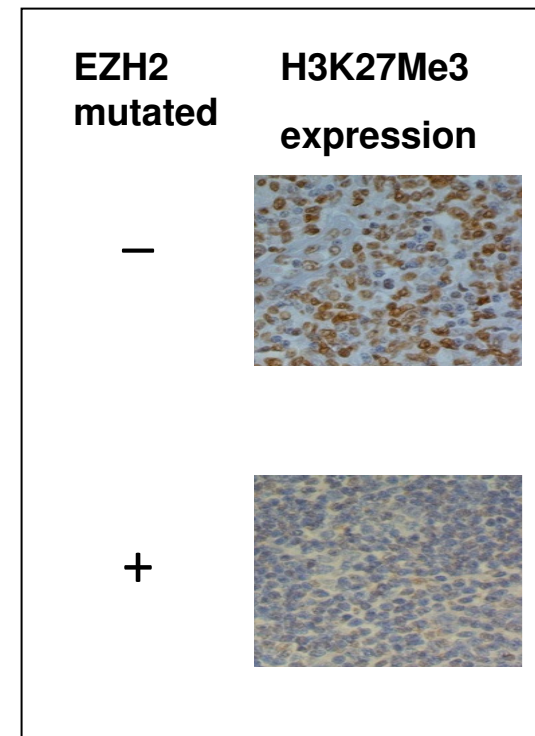
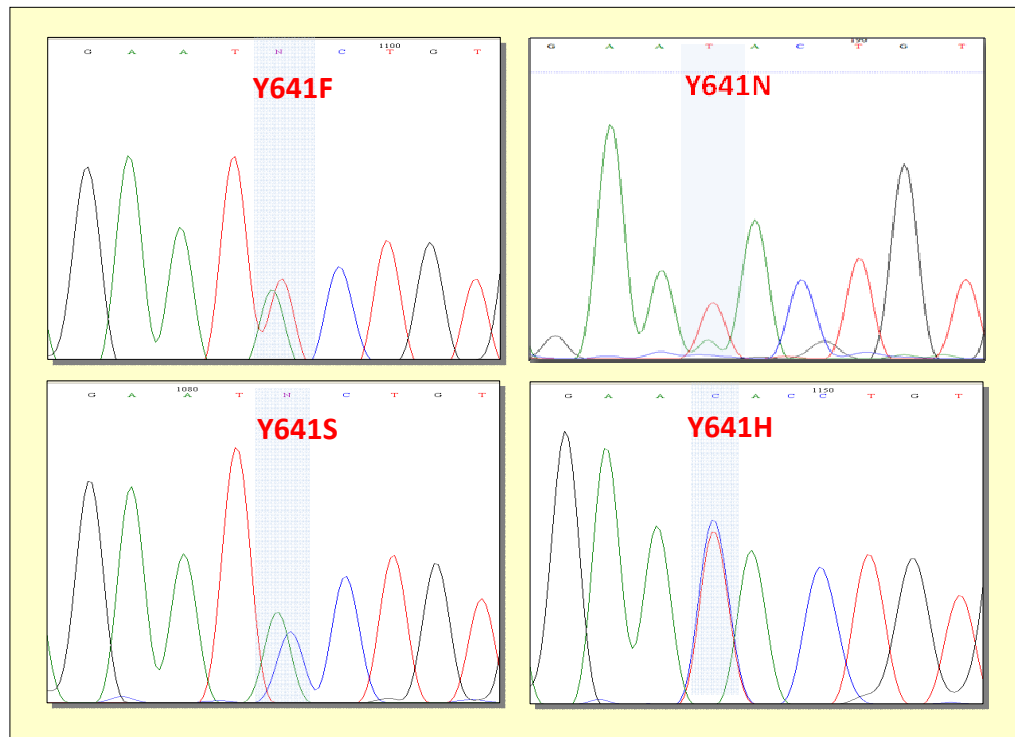
EZH2 Y641 mutated in FL (Vancouver – Morin 10)

- 225 FL : 26 mutations (11%)....Barts series
- 20 FL – tFL pairs: 30%
- Y641F (14), Y641N (8), Y641H (3), Y641S (1)



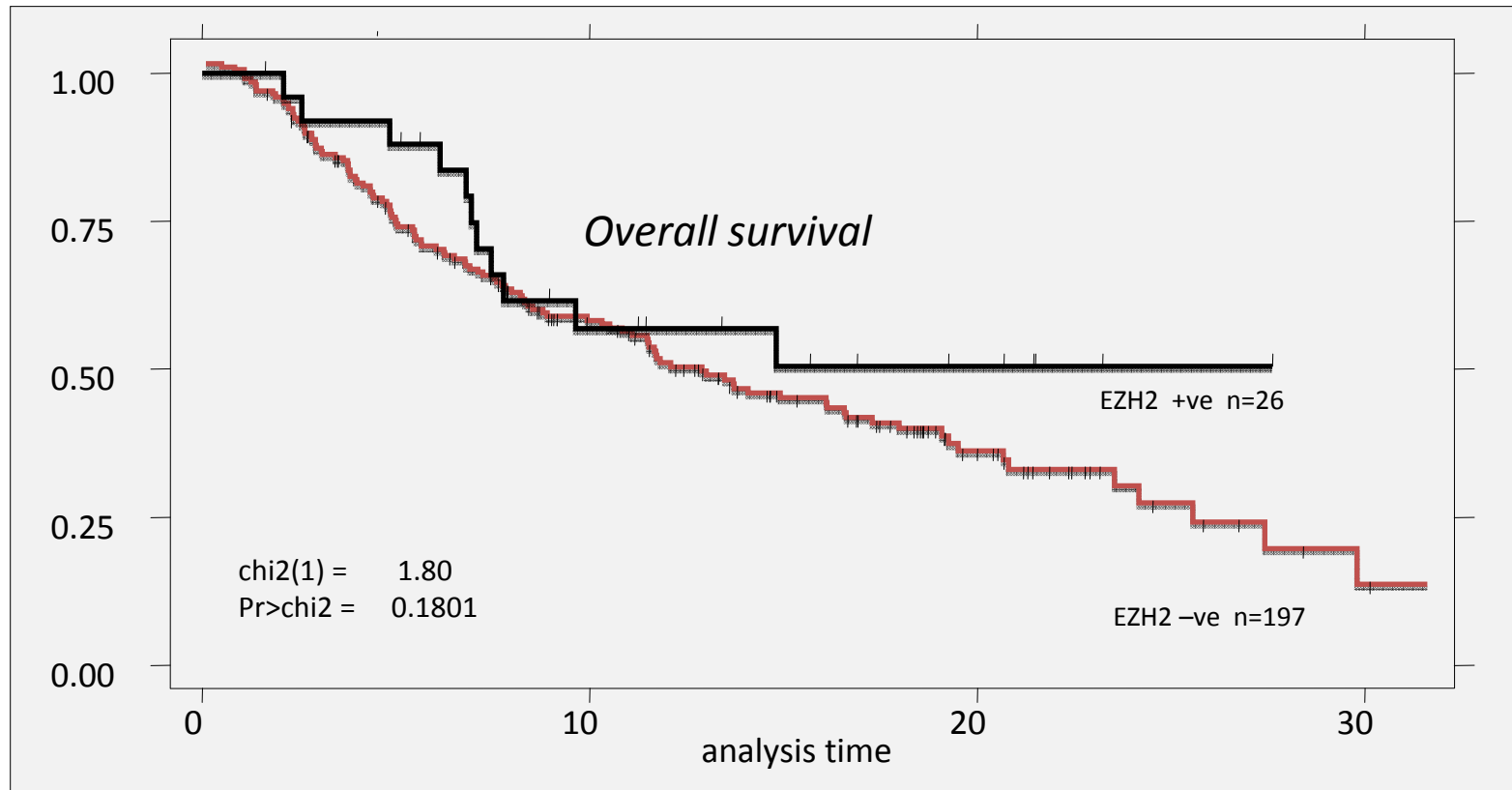
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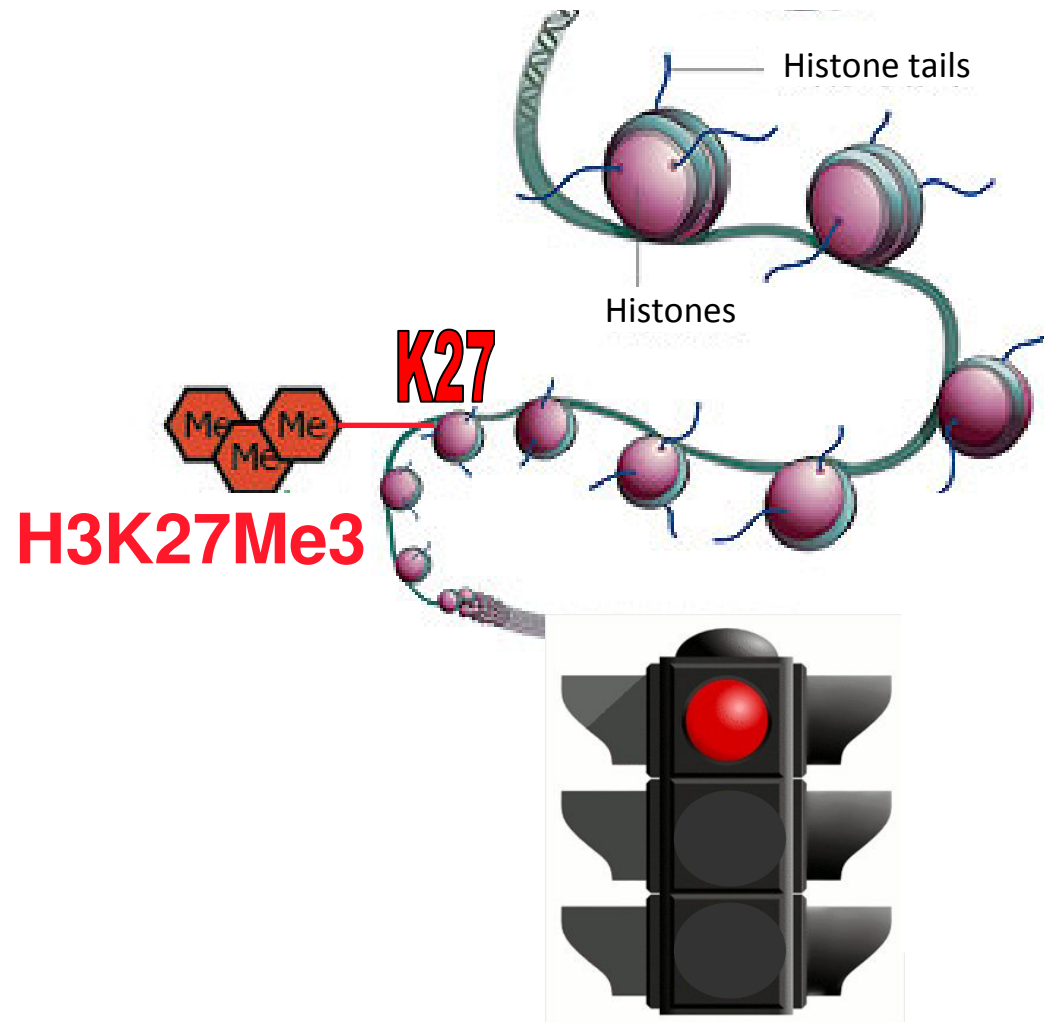
Mechanisms other than mutation - >30%

EZH2 Y641 not linked with outcome



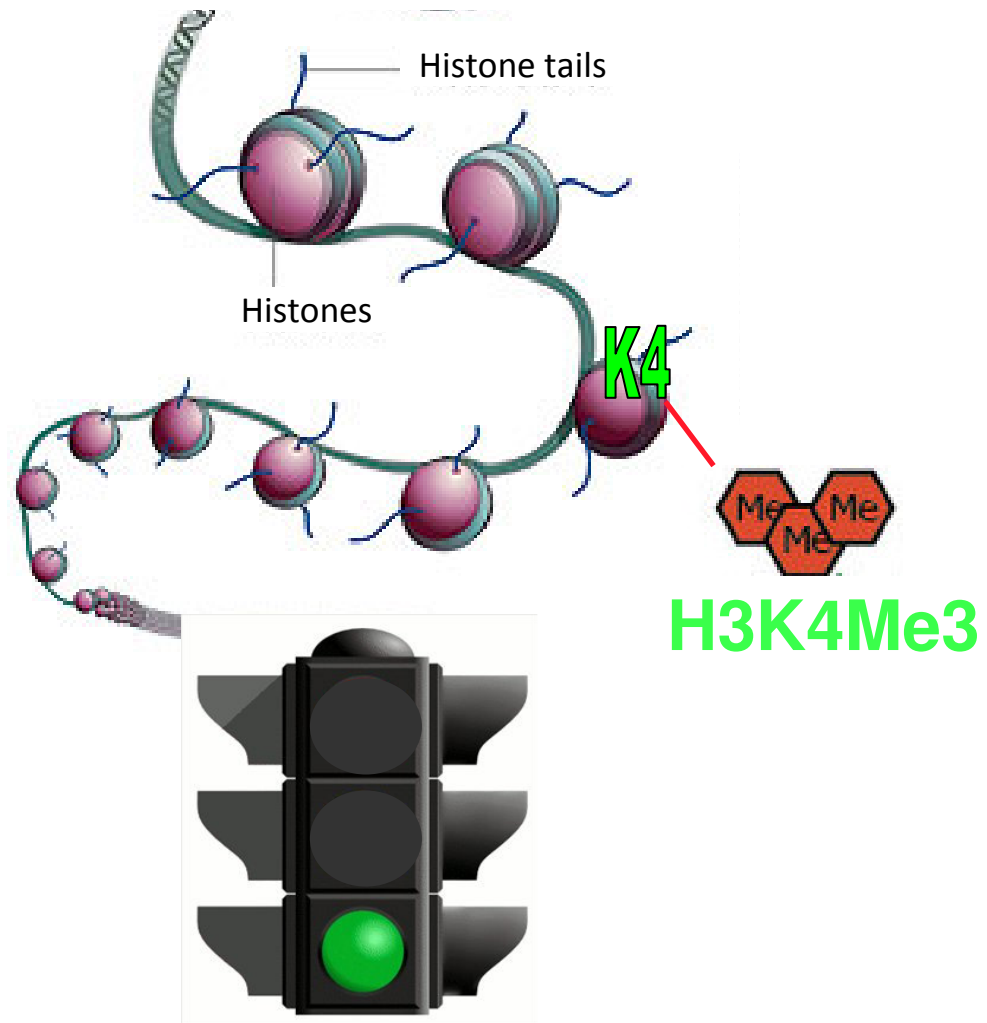
Histone Methyl marks (K4, K27)

- Gene expression **stopped**



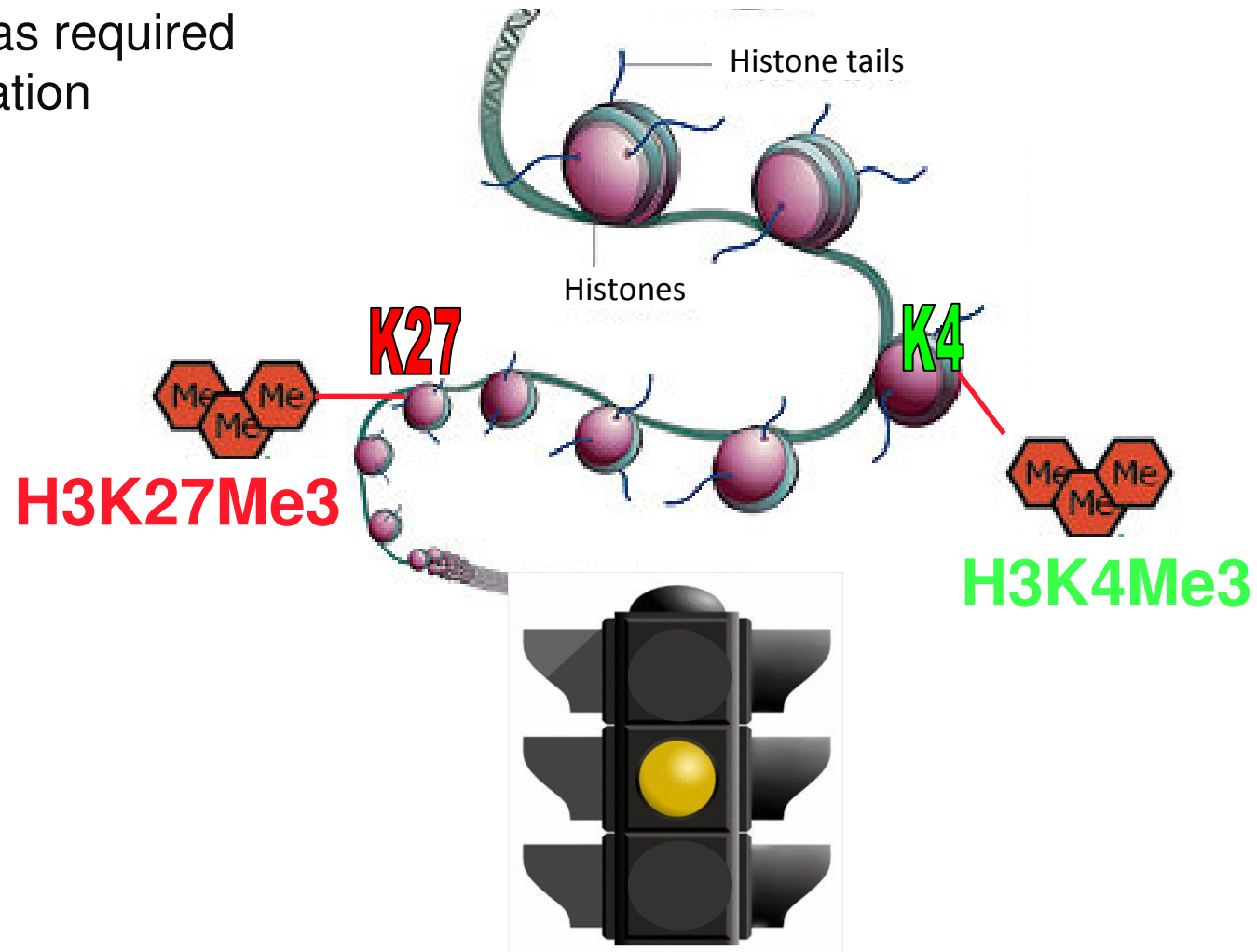
Histone Methyl marks (K4, K27)

- Gene expression **ON**

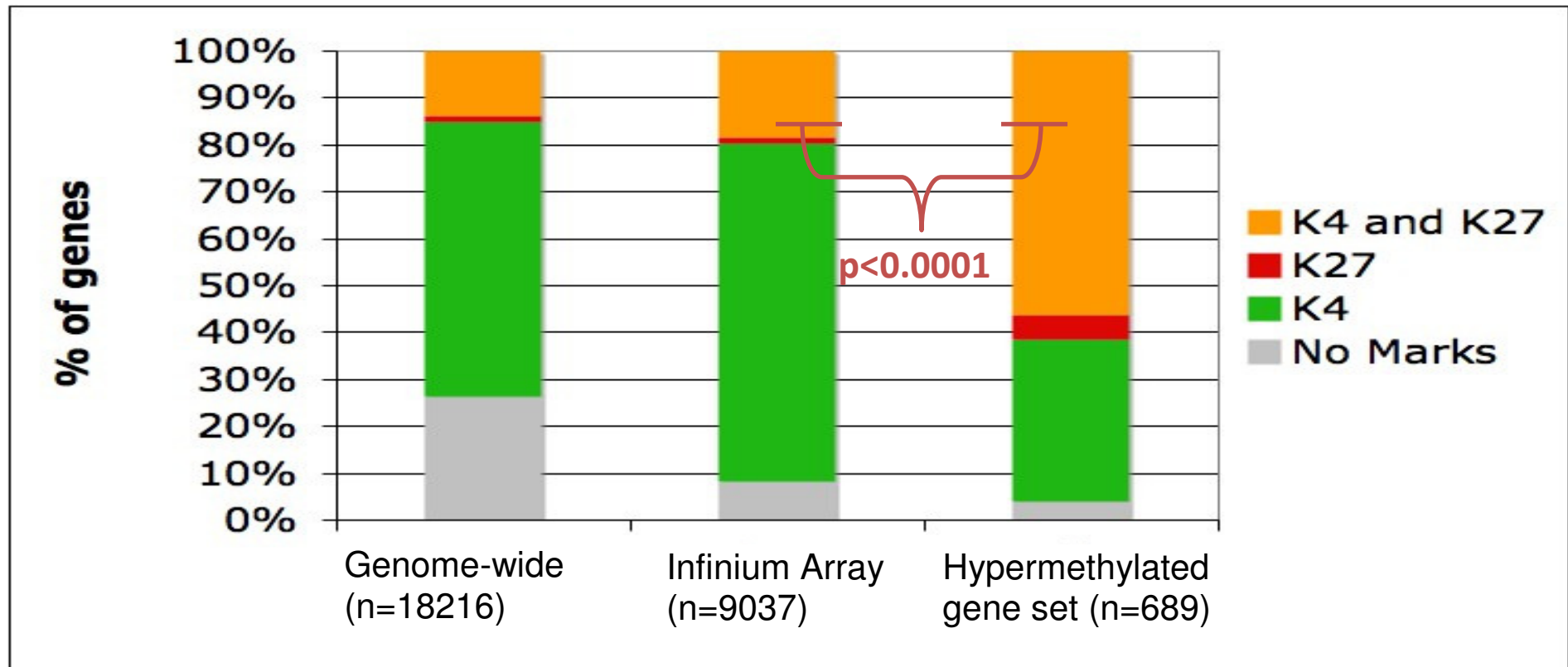


Histone Methyl marks (K4, K27)

- State of **readiness**
- Lose marks as required for differentiation



Enrichment for genes with bivalent domains in ES cells



Based on mapping by :Ku et al., PLoS Genetics 2008

Data on frequency of Histone modifications.

	Hypermethylated		Infinium Array		Genome Wide	
	n=	%	n=	%	n=	%
No Marks	28	4.1	737	8.2	4600	26.2
K4	233	34.1	6524	72.2	10218	58.3
K27	37	5.4	116	1.3	213	1.2
K4 and K27	386	56.4	1660	18.4	2510	14.3
<i>TOTAL</i>	<i>684</i>	<i>100</i>	<i>9037</i>	<i>100</i>	<i>17541</i>	<i>100</i>

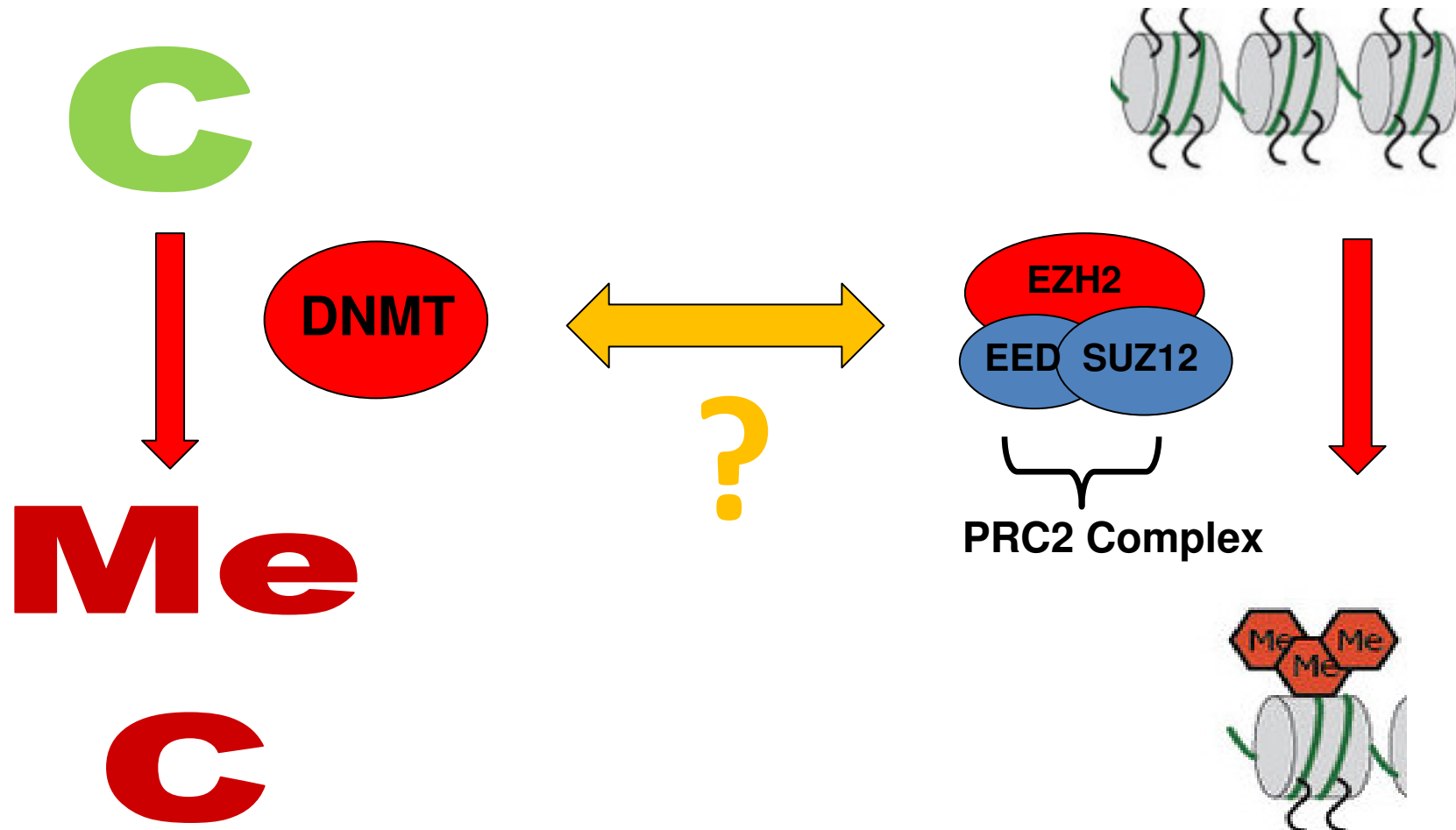
Gene Ontology (GO) in hypermethylated gene set.

GO term	Hypermethylated genes (%)	Infinium array set (%)	Adjusted P value
Multicellular organismal development	38.1	18.5	2.05×10^{-25}
Anatomical structure development	34.5	17.2	2.79×10^{-21}
System development	30.4	14.4	3.01×10^{-19}
Nervous system development	18.45	7.1	1.18×10^{-14}
Cell-cell signalling	15.1	5.5	8.77×10^{-14}
Organ development	22.3	10.9	2.46×10^{-11}

DNA Methylation

vs

Histone Methylation



Both can be pharmacologically manipulated !

Azacytidine

DZNep

Summary

- Aberrant hypermethylation in >700 genes in FL
- Early pre-programmed methylation of large number of development-related genes
- Occurs in approximately 8% of CpG islands
- Genes marked by bivalent domains in ES cells
- Wide variability in immunohistochemical H3K27Me3 expression in FL
- Frequent mutation of histone methylase EZH2 in FL

Current Plans

- To test methylation profile as an outcome predictor (choosing high tumour burden biopsies – 600 cases).
- Determine the effects of EZH2 Y641 mutation on clinical outcome.
- To correlate EZH2/H3K27me3 promoter occupancy with DNA methylation in order to identify methylation dependent and independent PRC2 target genes
- Determine the effects of EZH2/H3K27m3 and DNA methylation inhibition on primary and FL cell lines.
- Other mechanisms regulating H3K27me3

Acknowledgements

- **Centre for Medical Oncology**

Ciaran O' Riain

Csaba Bodor

T Andrew Lister



- **Genome Centre, Queen Mary University of London**

Charles Mein

Acknowledgements

