



The Way Ahead.™

Methyl-DIP on an Affymetrix chip

James Flanagan, PhD

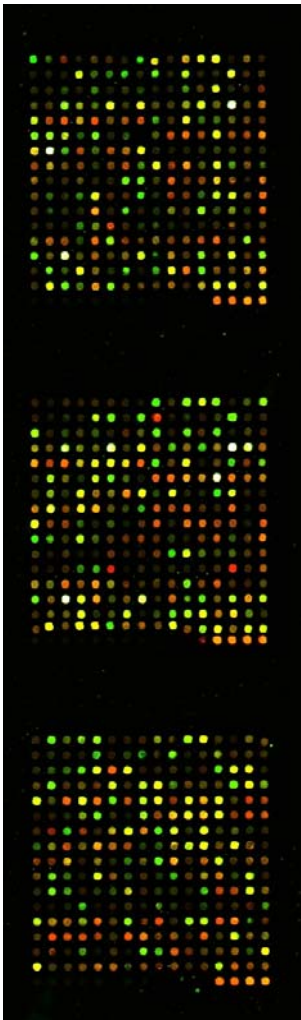
Wolfson Institute for Biomedical Research, UCL



1st European ChIP-on-chip Scientific Forum



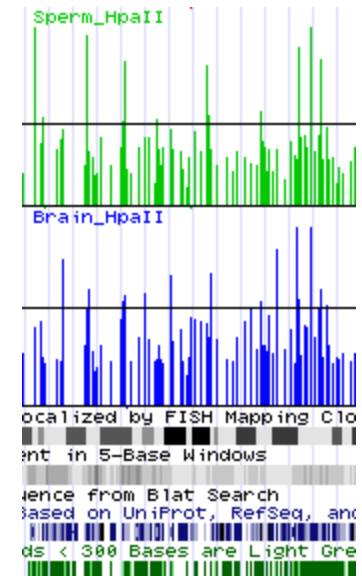
Methylation Microarrays by MS-RE digest



- Flanagan et al, *AJHG* 2006



- Hypothesis : *Epigenetic variation in the germ line is one of the many factors determining the phenotypic variation in normal and disease*
- <http://www.epigenomics.ca>



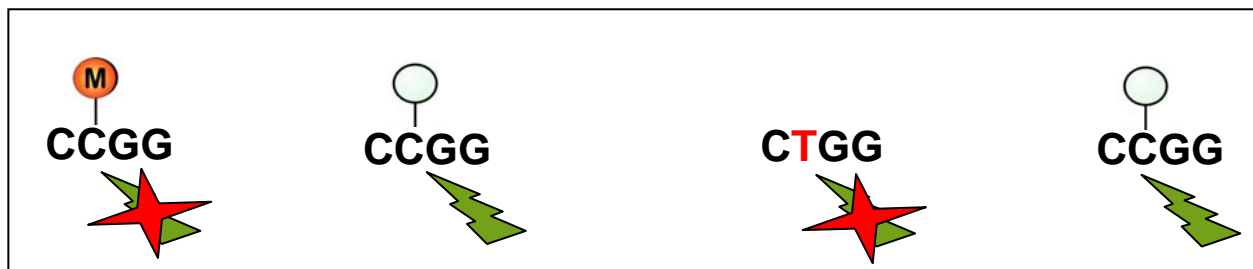
Other Methylation Microarray Methods

- **Weber et al, *Nature Genetics* 2005**
 - **Met-C antibody**
- Lippman et al, *Nature Methods* 2005
 - McrBC restriction enzyme
- Hatada et al, *Oncogene* 2006
 - HpaII vs MspI restriction enzyme
- Schumacher et al, *Nuc. Acids Research* 2006
 - HpaII + Hin6I + AclI restriction enzyme
- Gitan et al, *Genome Research* 2001
 - Bisulphite treated DNA approach

Rationale for (not) choosing MS-RE

- Confounding factors

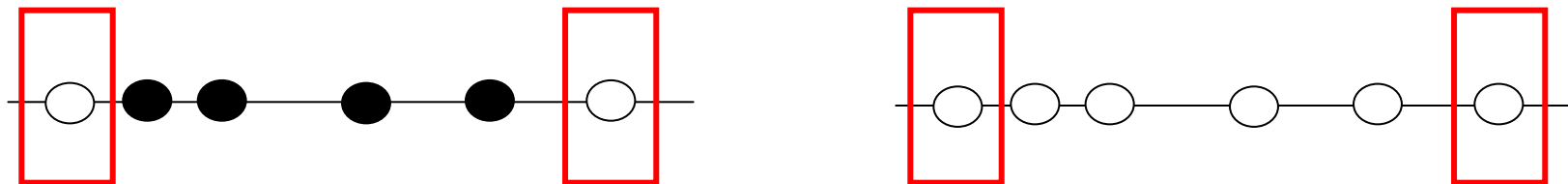
- SNPs



- Mutations, LOH, CNVs, Ins/Del

- Validation of array results

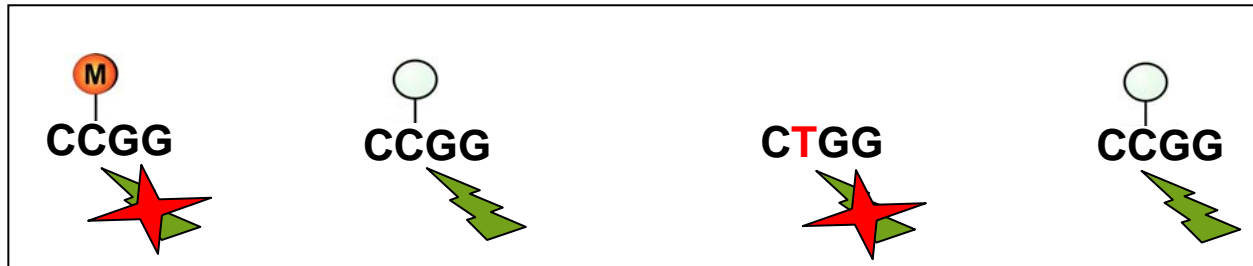
- Only the restriction enzyme site can/should be validated !



Rationale for (not) choosing MS-RE

- Confounding factors

- SNPs



- Mutations, LOH, CNVs, Ins/Del

- Validation of array results

- Only the restriction enzyme site can/should be validated !

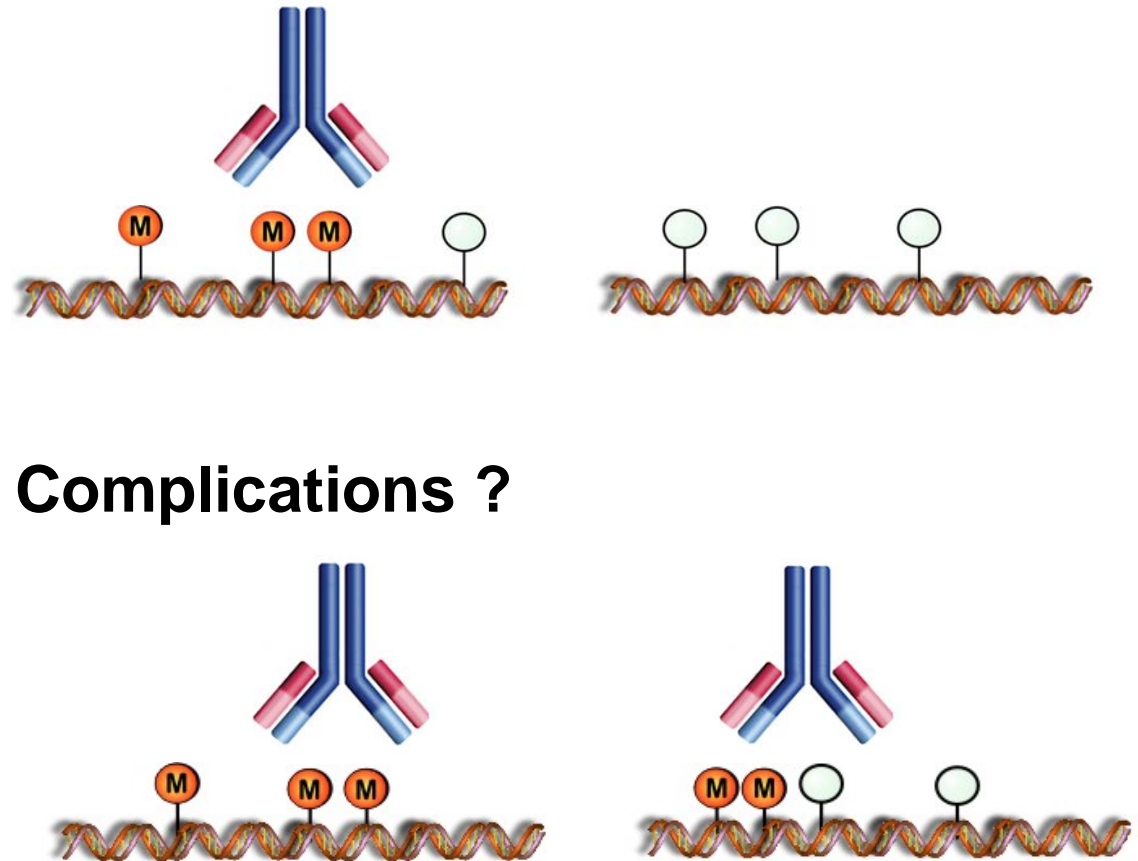
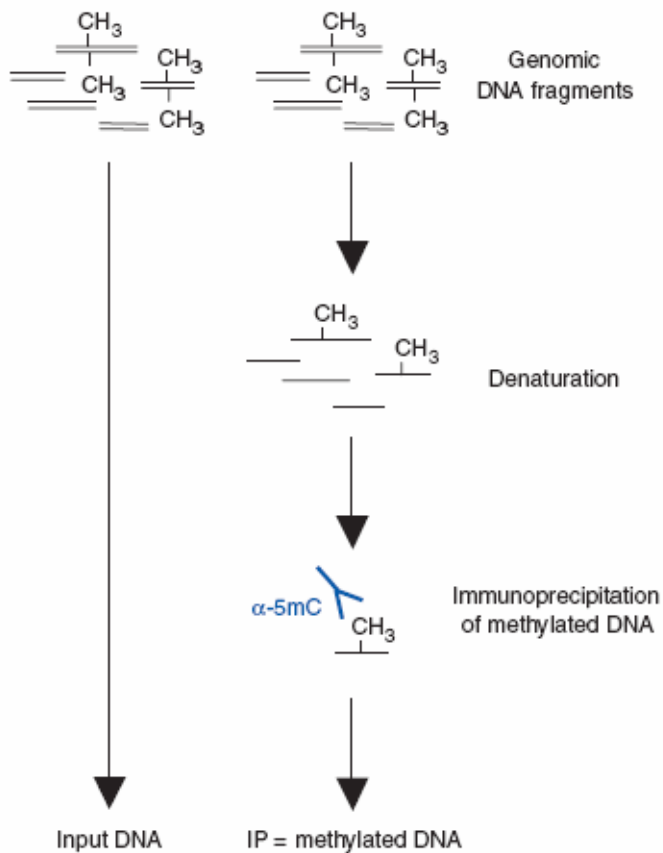


Rationale for choosing Methyl-DIP

- Can be performed on one sample
 - Input vs Antibody bound
- Potentially quantitative methylation percentage
 - Higher signal (enrichment) = higher percent methylation
- Results are much more comparable to other ChIP-chip experiments
 - Eg. Histone modifications, TF binding site

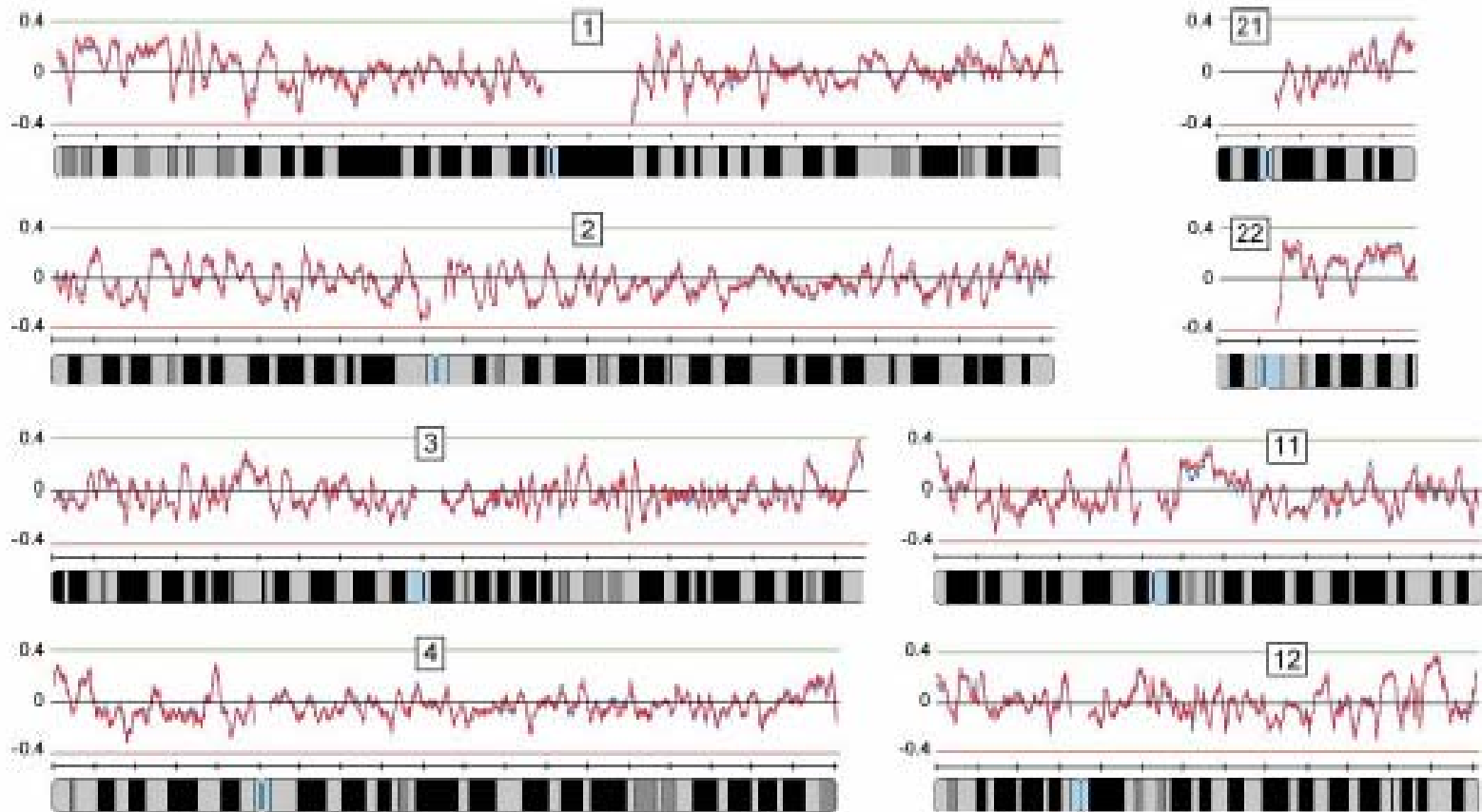
Methyl-DIP – Weber et al *Nat Genet.* 2005

a

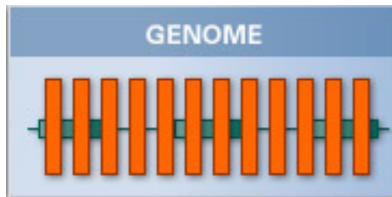


Methyl-DIP – Weber et al *Nat Genet.* 2005

Genome wide methylation in primary fibroblasts using BAC tiling array

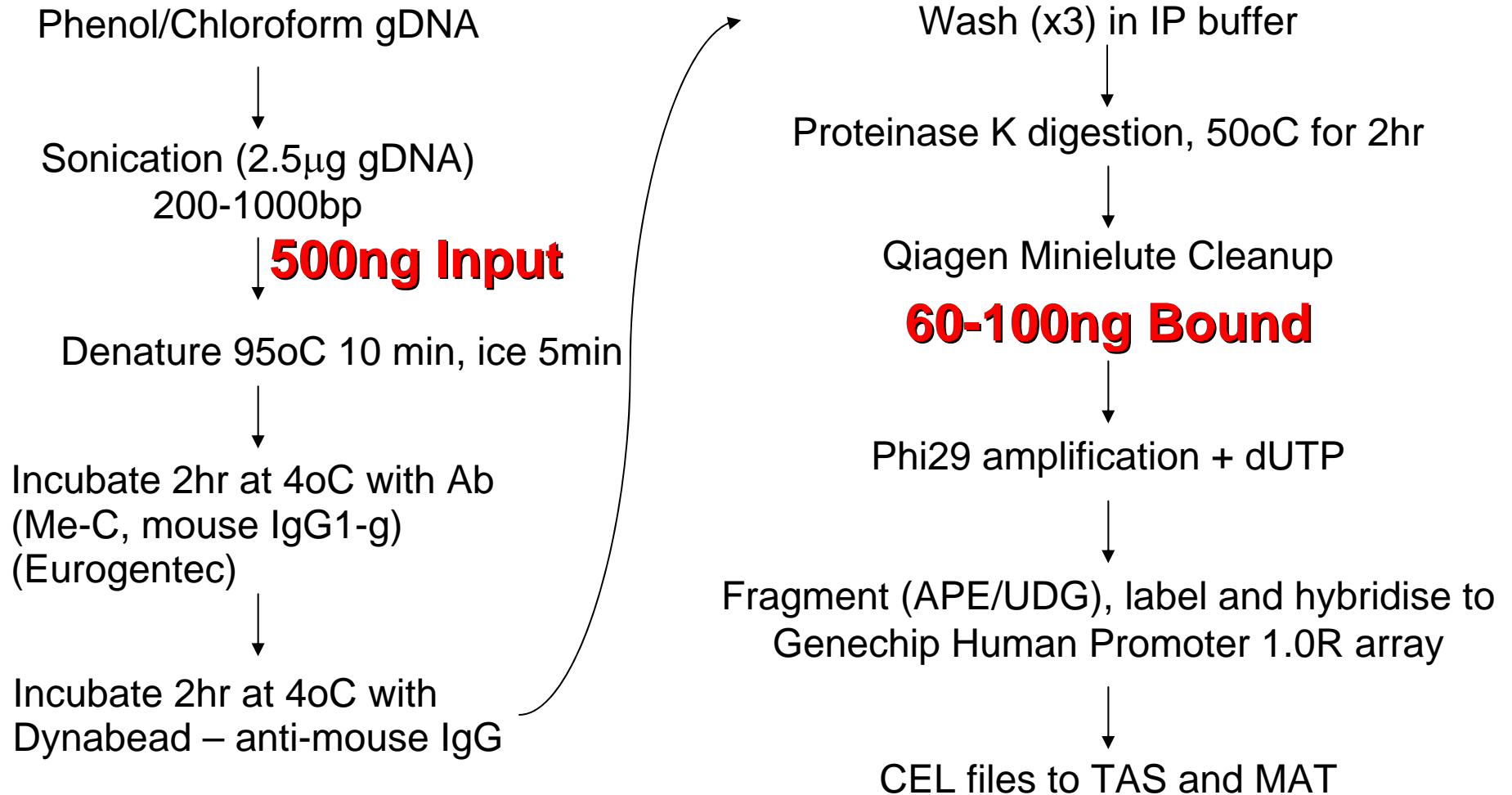


Genechip Human Promoter 1.0R array

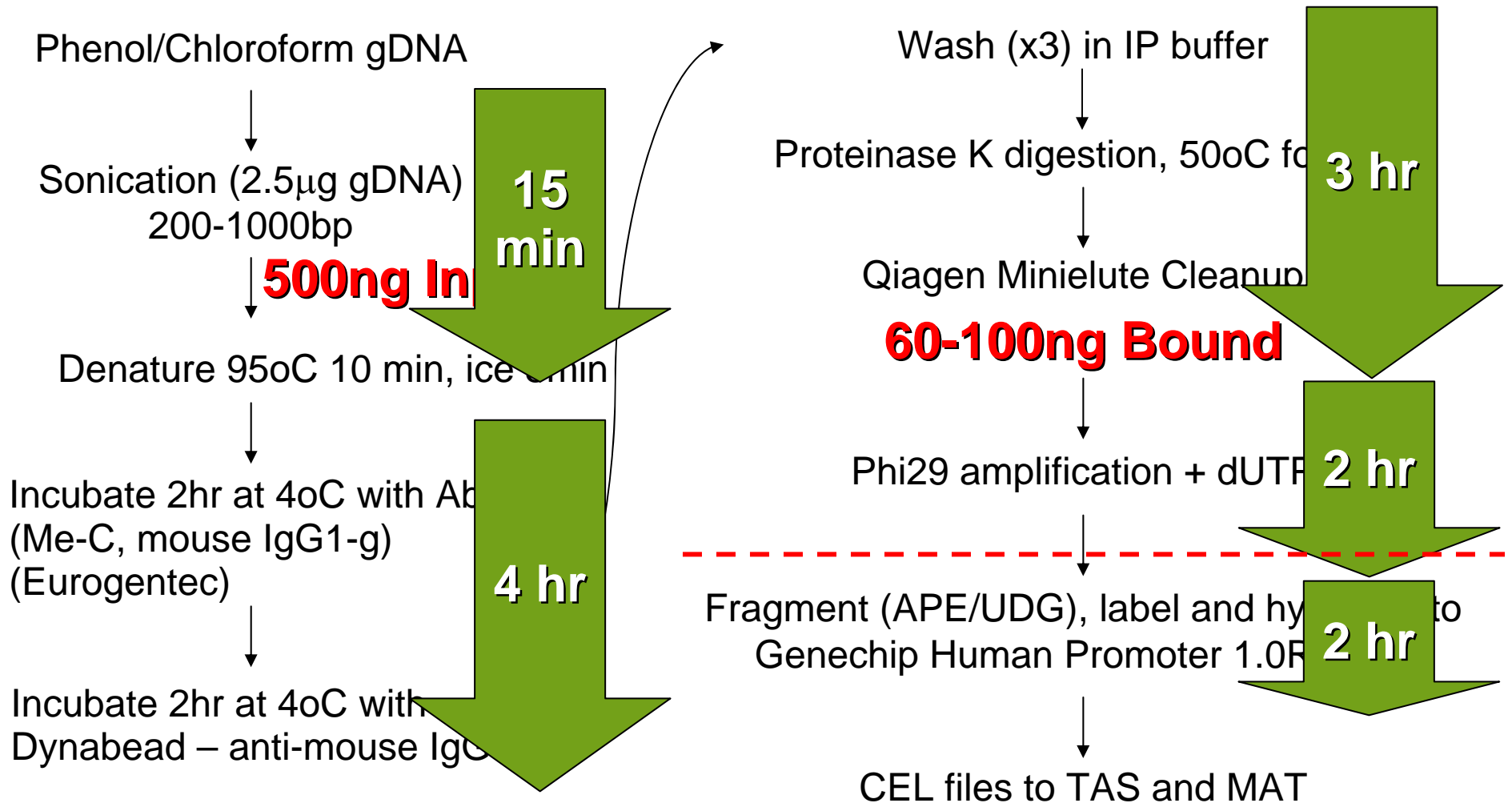


- Single array for investigation of 25,500 human promoter regions.
- 4.6 million probes tiled over ~10kb for each promoter.
- 25-mer probes tiled with 35bp spacing.
- a subset of the probes used in the whole-genome GeneChip® Human Tiling 2.0R Array Set (P/N 900772).
- Repetitive elements were removed by RepeatMasker.

Methyl-DIP Workflow



Methyl-DIP Workflow



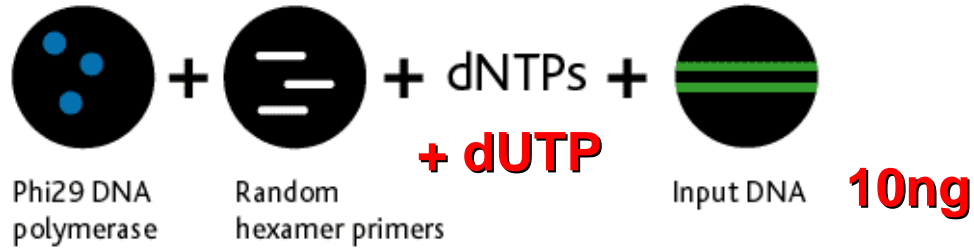


The Way Ahead.™

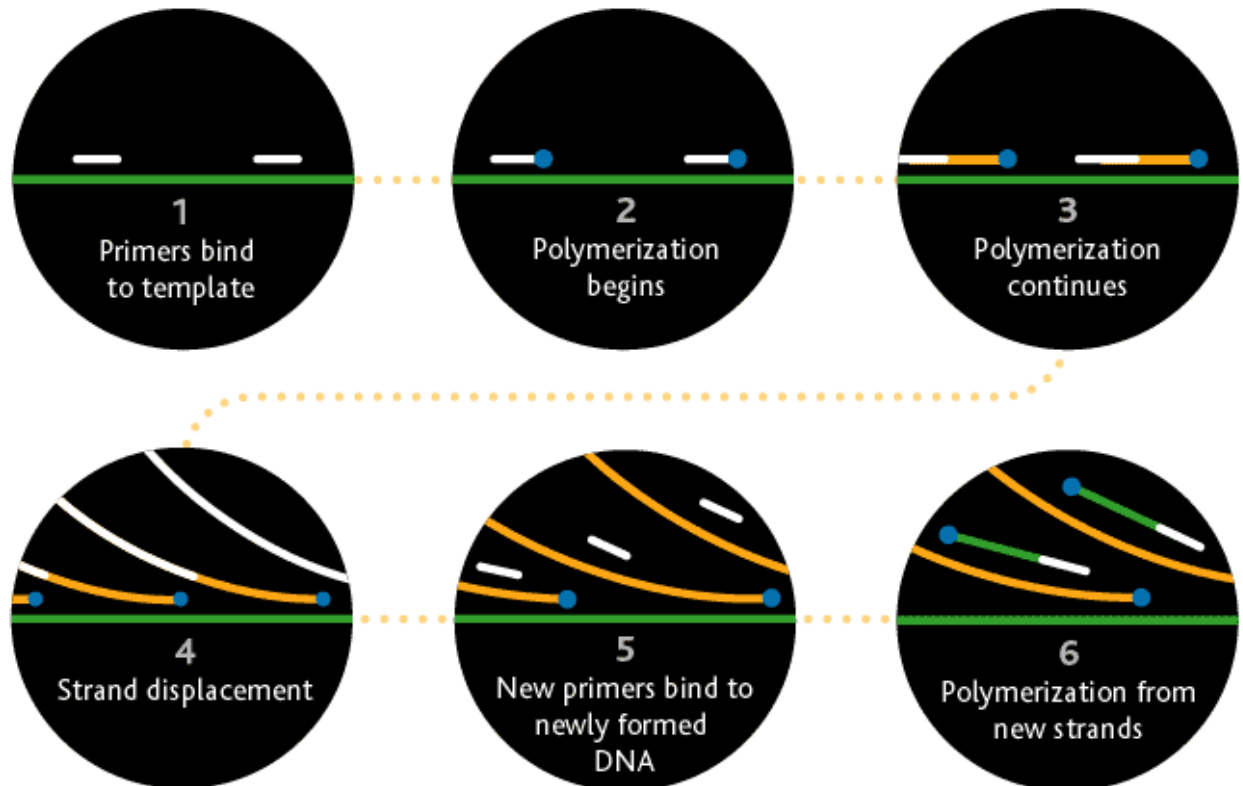
WGA - Genomiphi V2 for amplification



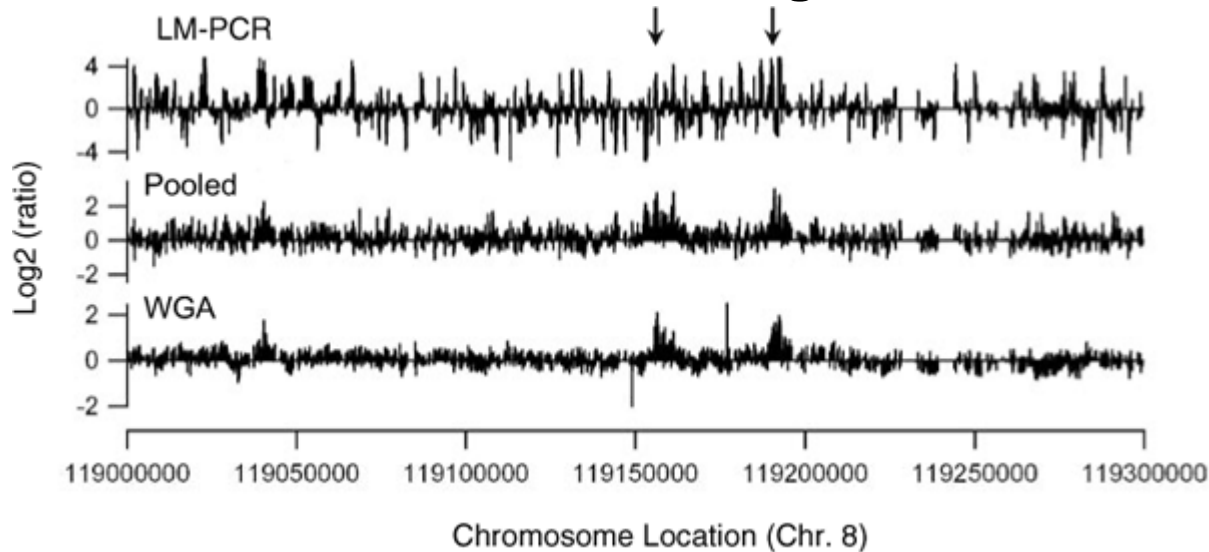
GE Healthcare



<http://www.gelifesciences.com>



- **ENCODE oligonucleotide arrays (NimbleGen)**
 - Duplicate arrays
- **Oct4 binding site ChIP**



Method	Total Peaks ^a	Overlapping ^b	Overlap (%)
LM-PCR	543	82	15
Pooled	491	343	70
WGA	449	280	63

LM-PCR, ligation-mediated PCR; WGA, whole genome amplification.
^aTotal number of peaks called on both arrays.
^bIf at least one of the ends of a peak region from one array overlapped a peak region from the other array, the peaks were considered to be overlapping.

LM-PCR = Ligation Mediated PCR

Pooled = pooling 10 individual ChIP samples

WGA = Genomplex (Sigma) Whole Genome Amplification & reamplification

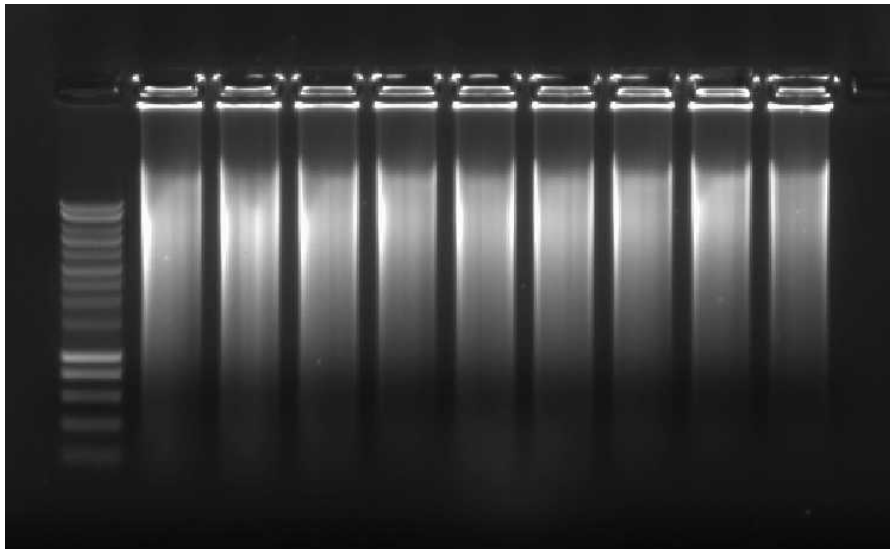
Methyl-DIP on Lymphatic Endothelial Cells

- Wang, Boshoff et al *Nat Genet* 2004
 - LEC gene expression is reprogrammed by KSHV
- Q. Which genes are methylated in LECs?
- Q. Does KSHV alter the methylation?
- Methyl-DIP on LECs using Genechip Human Promoter 1.0R array
 - Input (3 replicates) vs Bound (3 replicates)

Methyl-DIP on Lymphatic Endothelial Cells

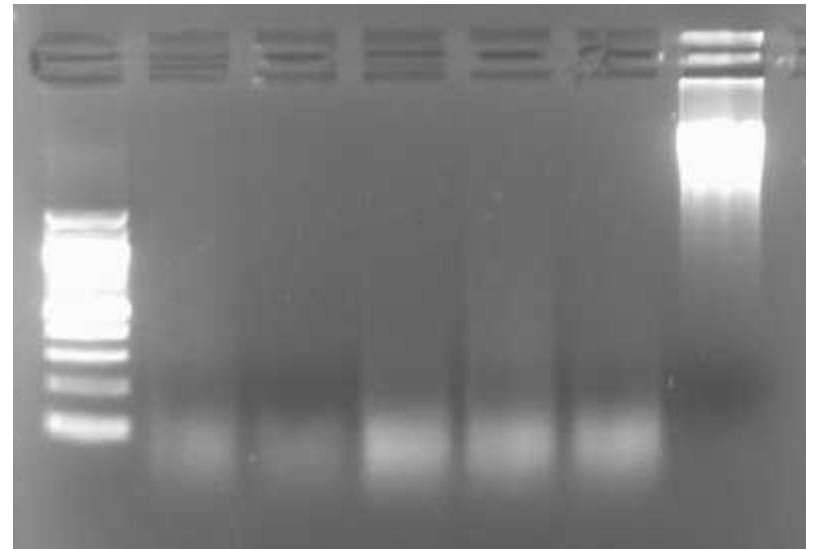
Genomiphi Amplification

Input Unbound Bound



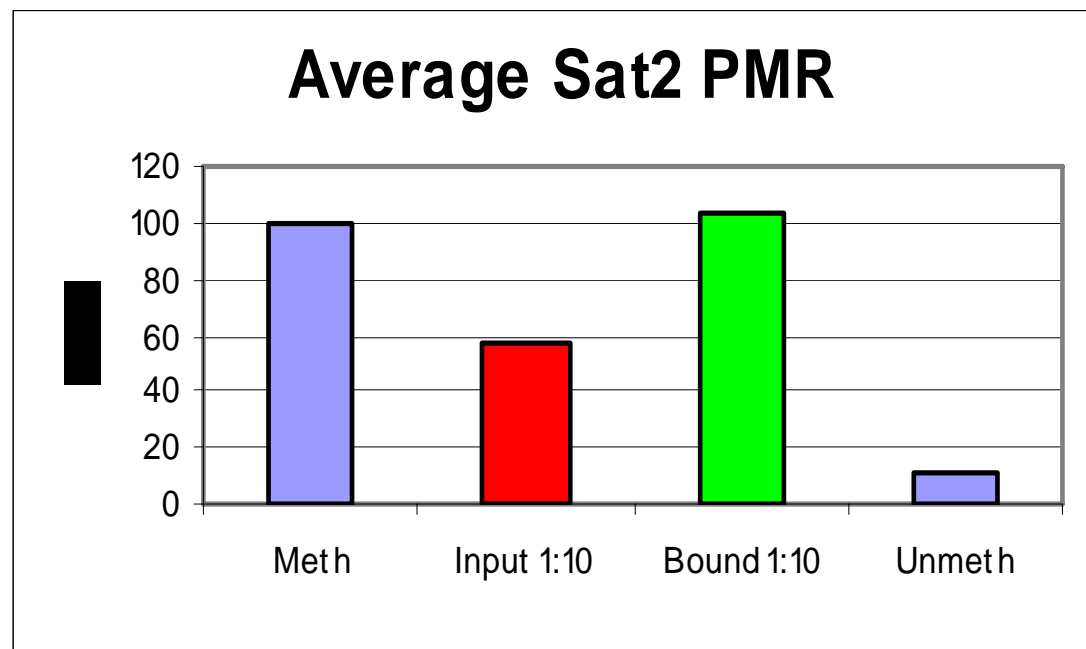
APE/UDG Fragmentation

I2 I3 B2 B3 B3



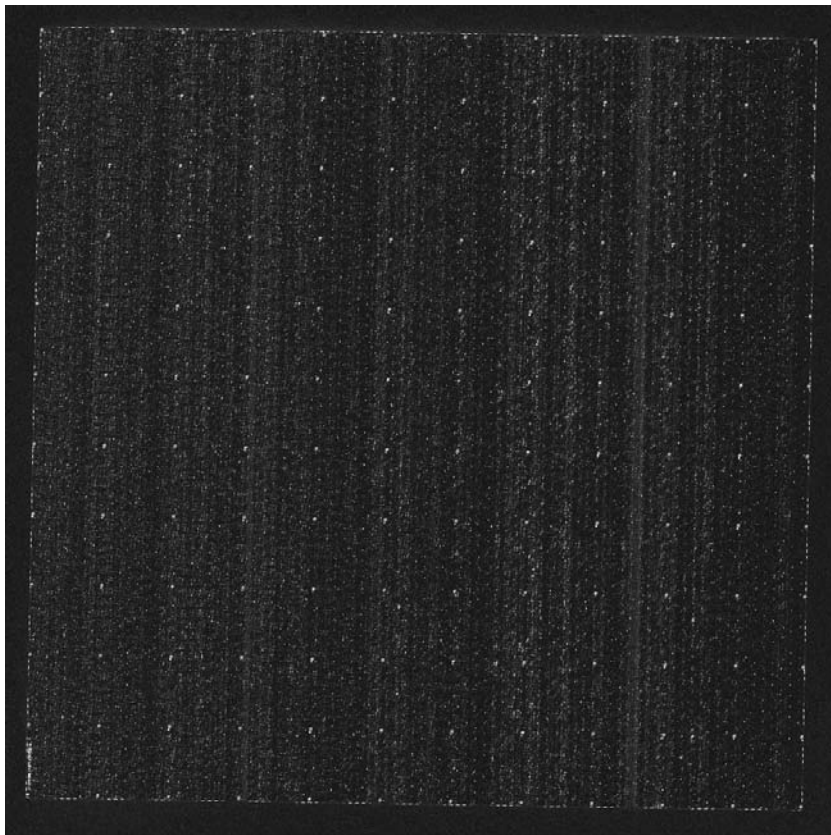
Methylight for Methyl-DIP efficiency

- Sat2 centromeric repeats are often methylated
 - Bisuphite treat Input and Bound LEC gDNA
 - Methylight assay for Sat2 and Alu-C loading control

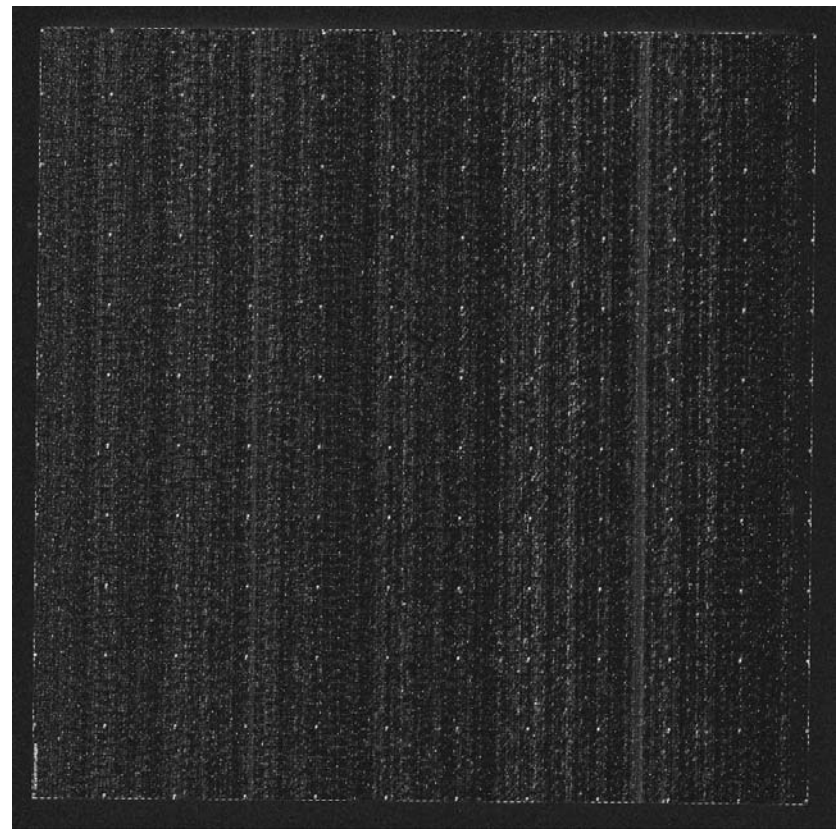


Results

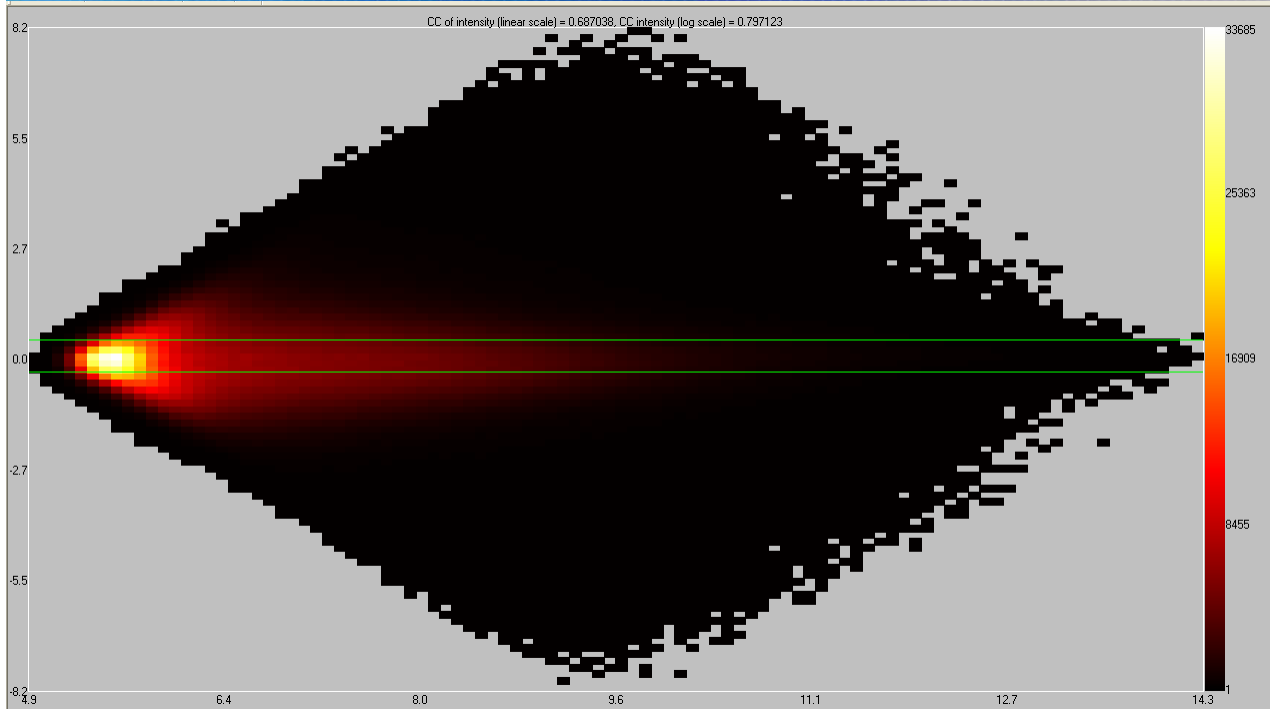
Input 3



Bound 3



MVA plots

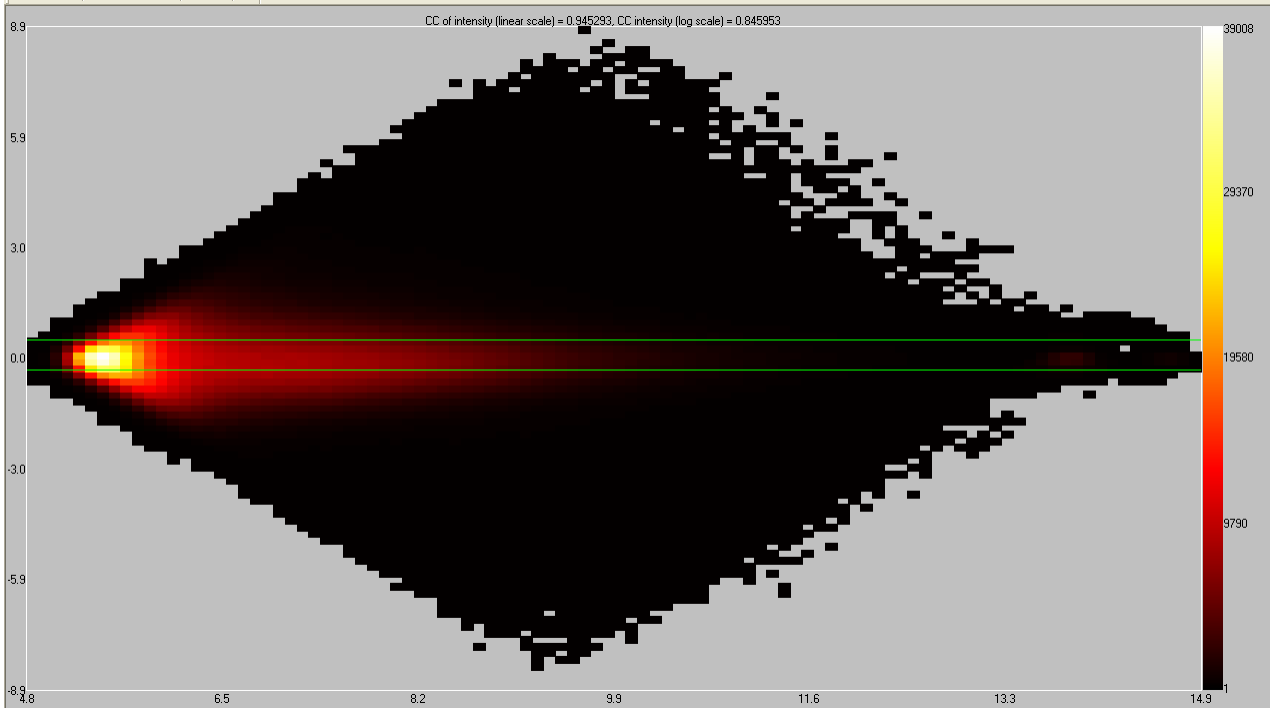


Bound 2
vs
Input 2



The Way Ahead.™

MVA plots

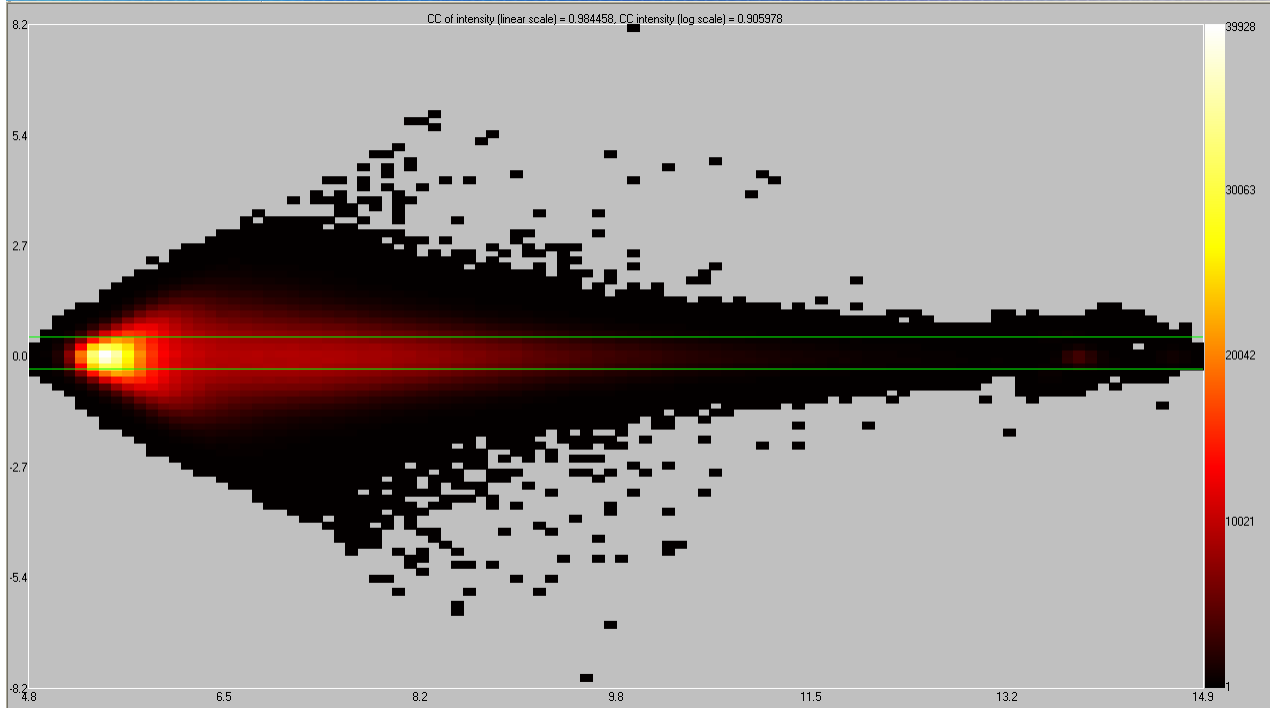


Bound 3
vs
Input 3



The Way Ahead.™

MVA plots



Input 2
vs
Input 3

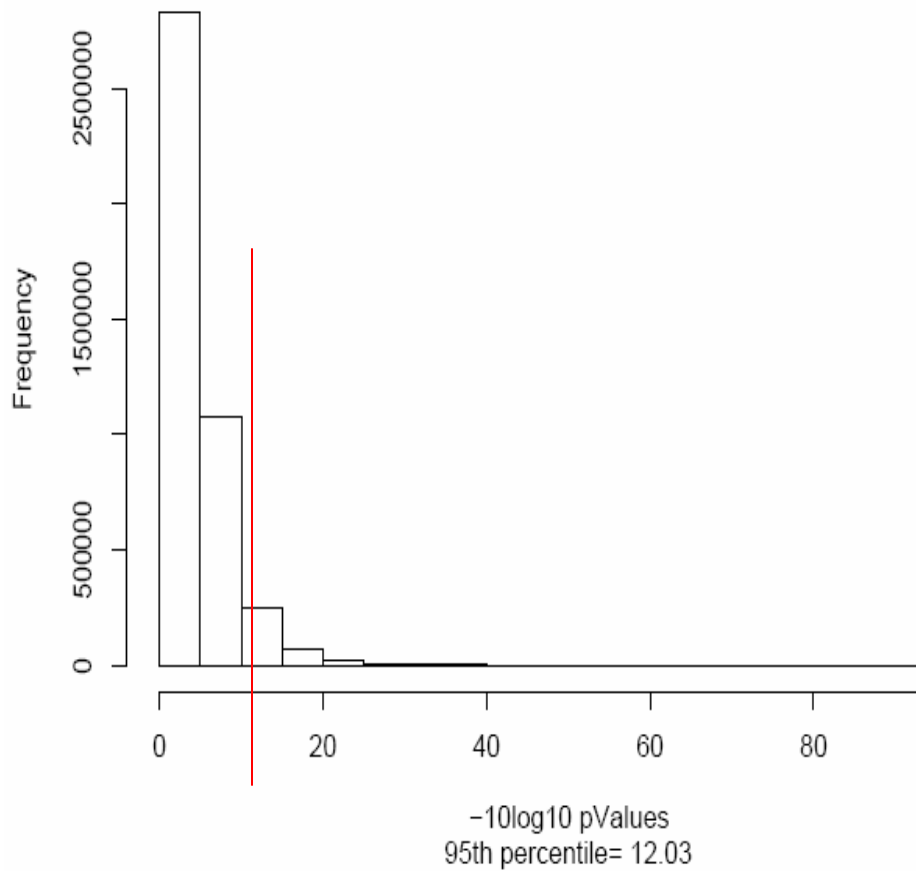
Tiling Array Software (TAS) analysis

- Peak detection (Interval Analysis, p-value)
 - Threshold = 13 ($p < 0.05$)
 - Max gap = 80bp (<3 probes)
 - Min run = 200bp (~ smallest fragment size)

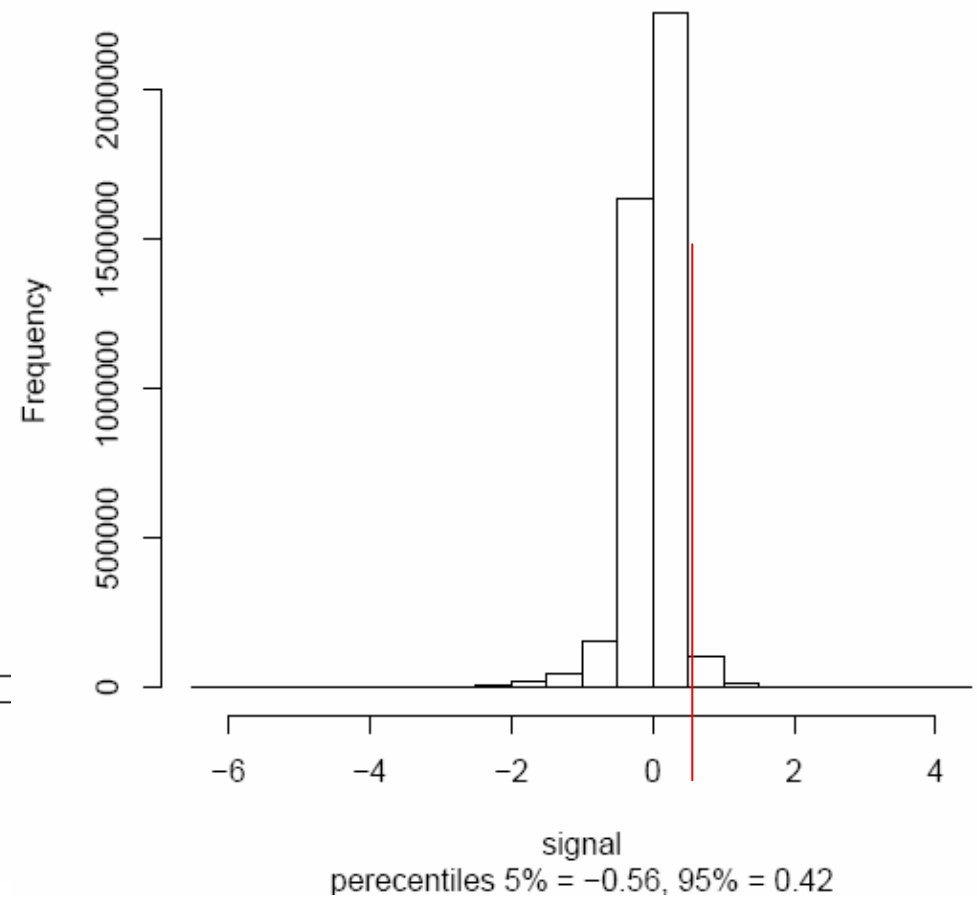
- Peak detection (Interval Analysis, log₂ intensity)
 - Threshold = >0.4 (> 1.3 fold signal change)
 - Max gap = 80bp (<3 probes)
 - Min run = 200bp (~ smallest fragment size)

P-values, Intensities Thresholds

Histogram of pv[, 2]



Histogram of sig[, 2]

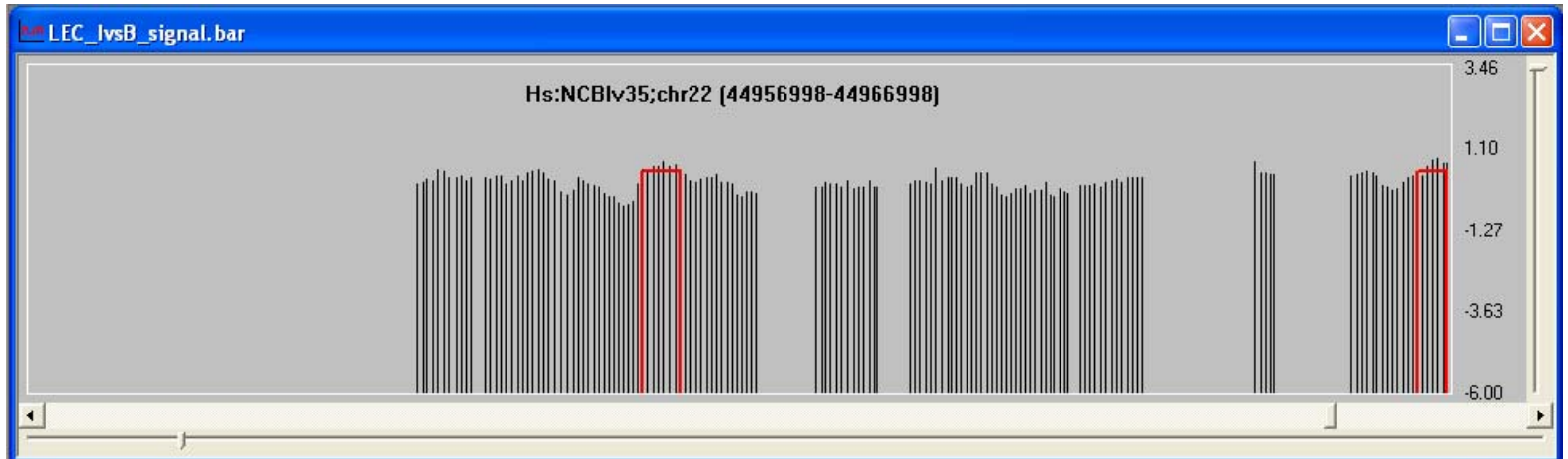


Using HEP for validation of methylated genes

- Human Epigenome Project
- Eckhardt et al. *Nature Genetics* Nov 2006
- Chr6, Chr20, Chr22
 - CD4, CD8 T cells, Fibroblasts, Keratinocytes, Melanocytes, Placenta, Embryonic Liver, Embryonic Skeletal Muscle, Heart, Liver, Skeletal Muscle and Sperm
 - 2524 amplicons
- Average methylation across all tissues
 - Chr 22, 718 amplicons with Avg Meth >60%
 - Chr 6, 342 amplicons with Avg Meth >60%

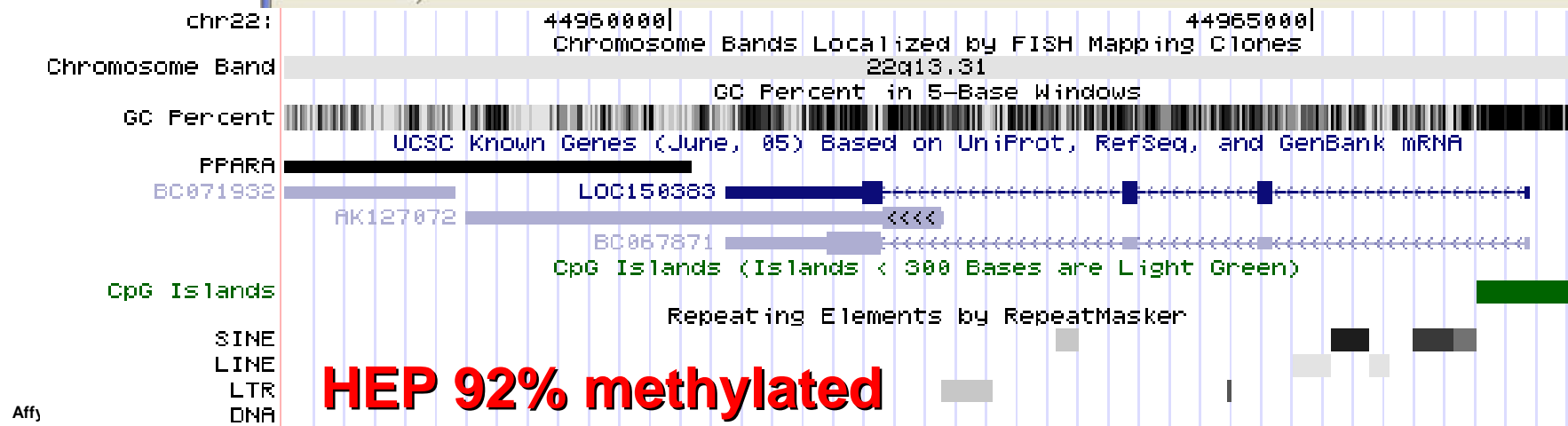
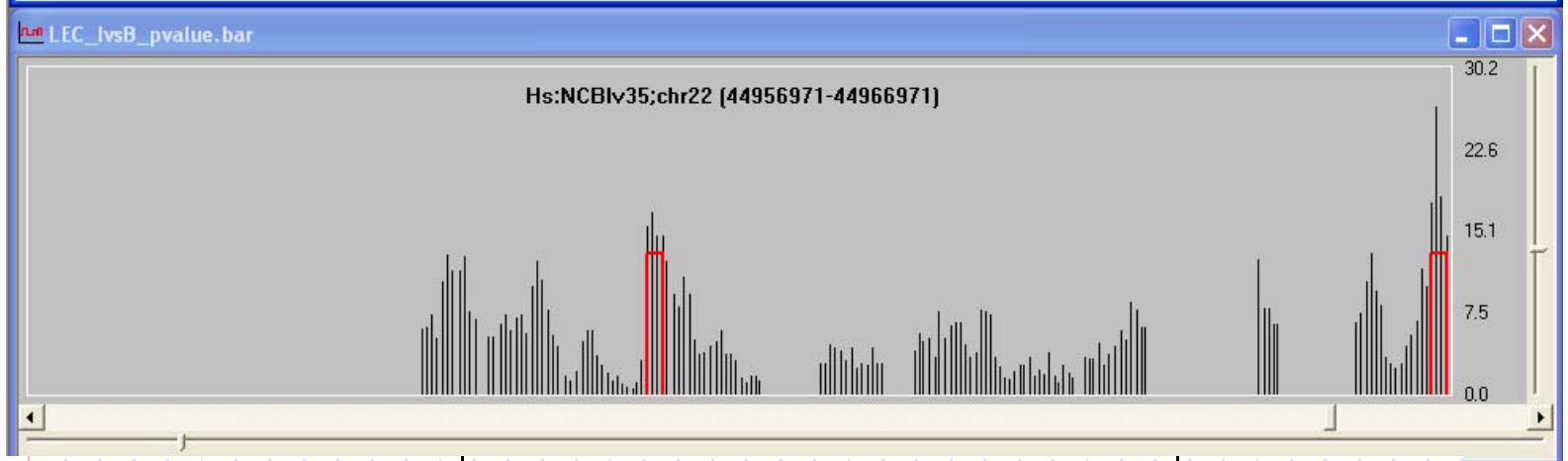
Intensity

Threshold = 0.4
Max Gap = 80bp
Min run = 200bp



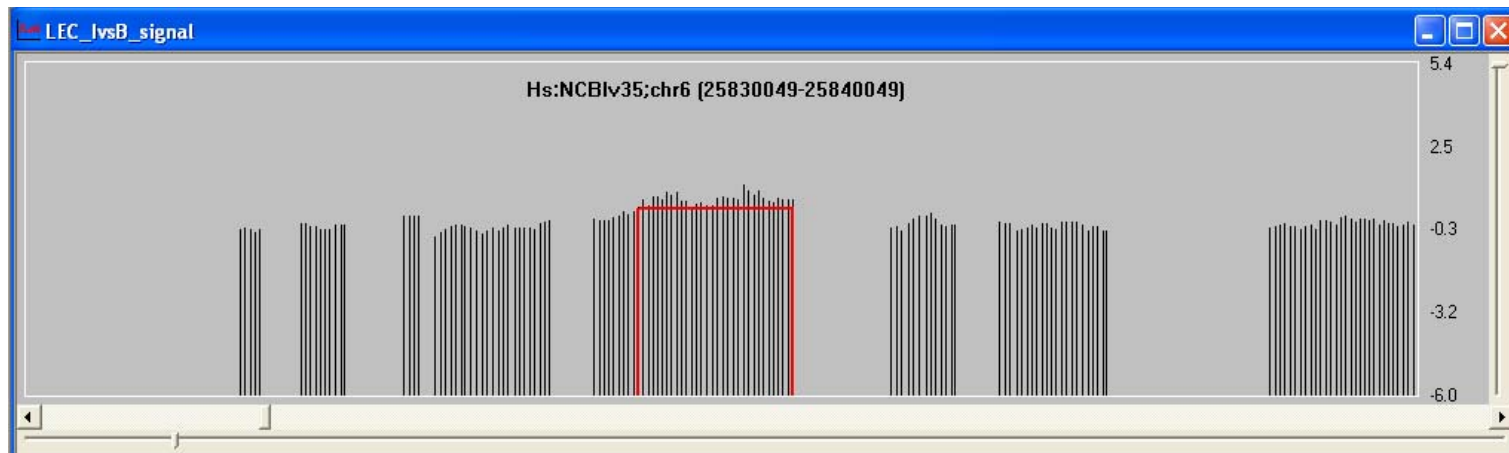
P-value

Threshold = 13
Max Gap = 80bp
Min run = 200bp



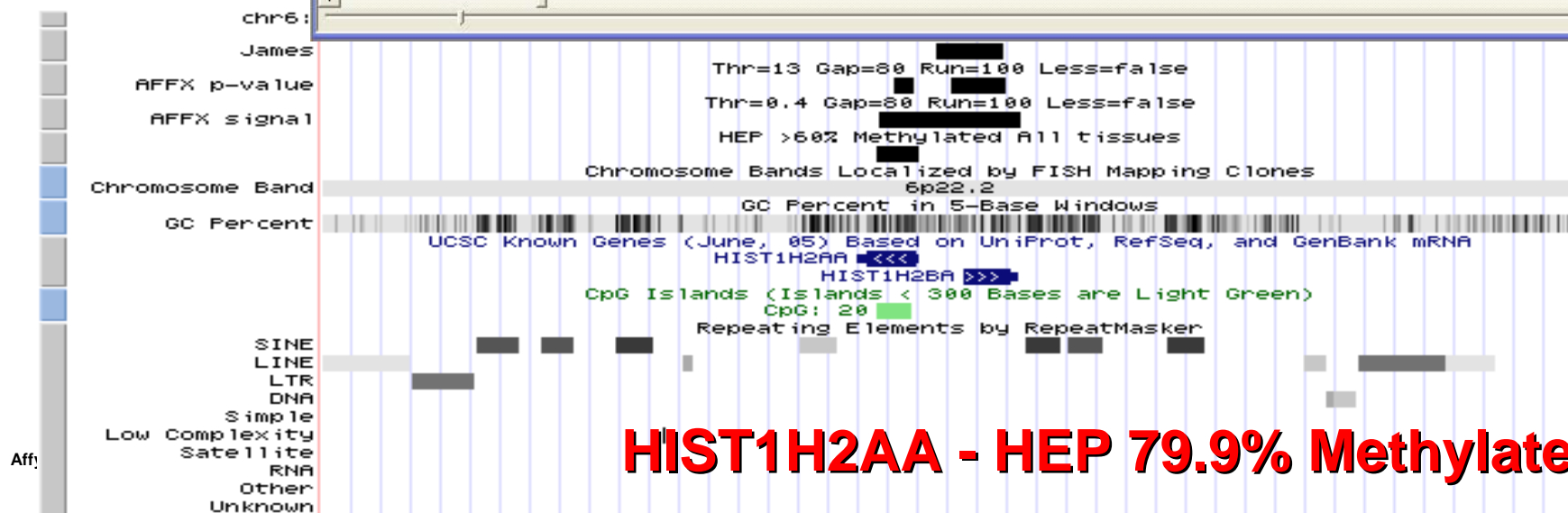
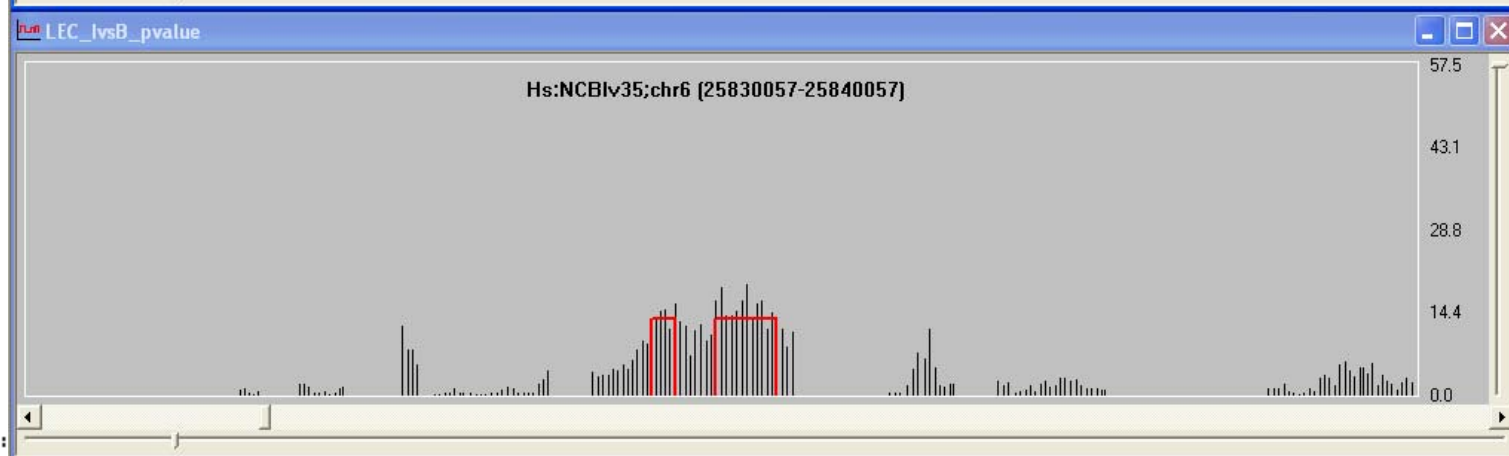
Intensity

Threshold = 0.4
Max Gap = 80bp
Min run = 200bp



P-value

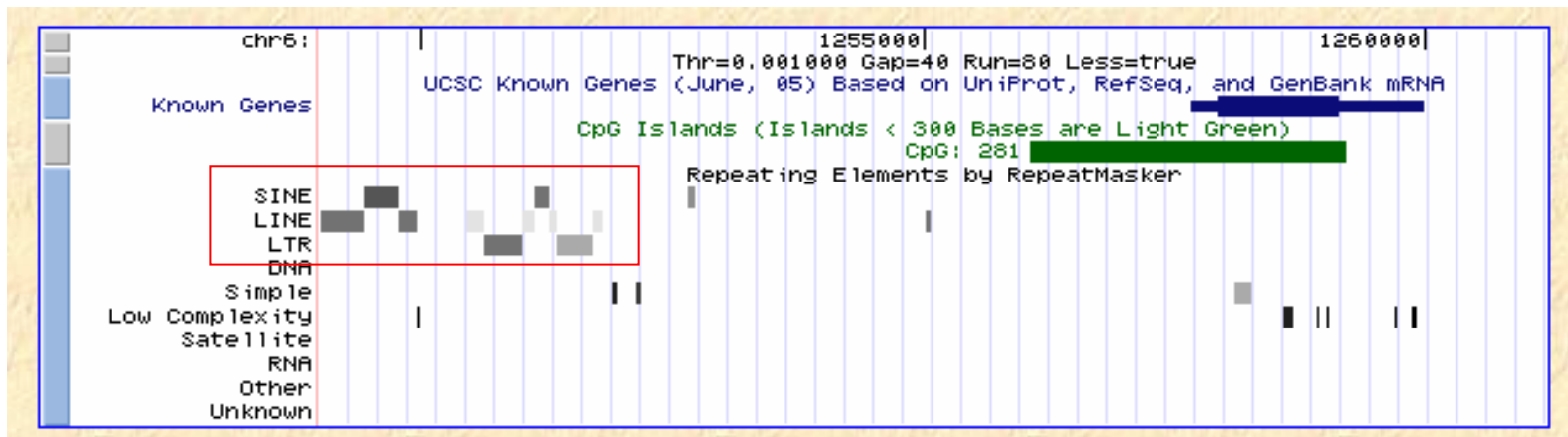
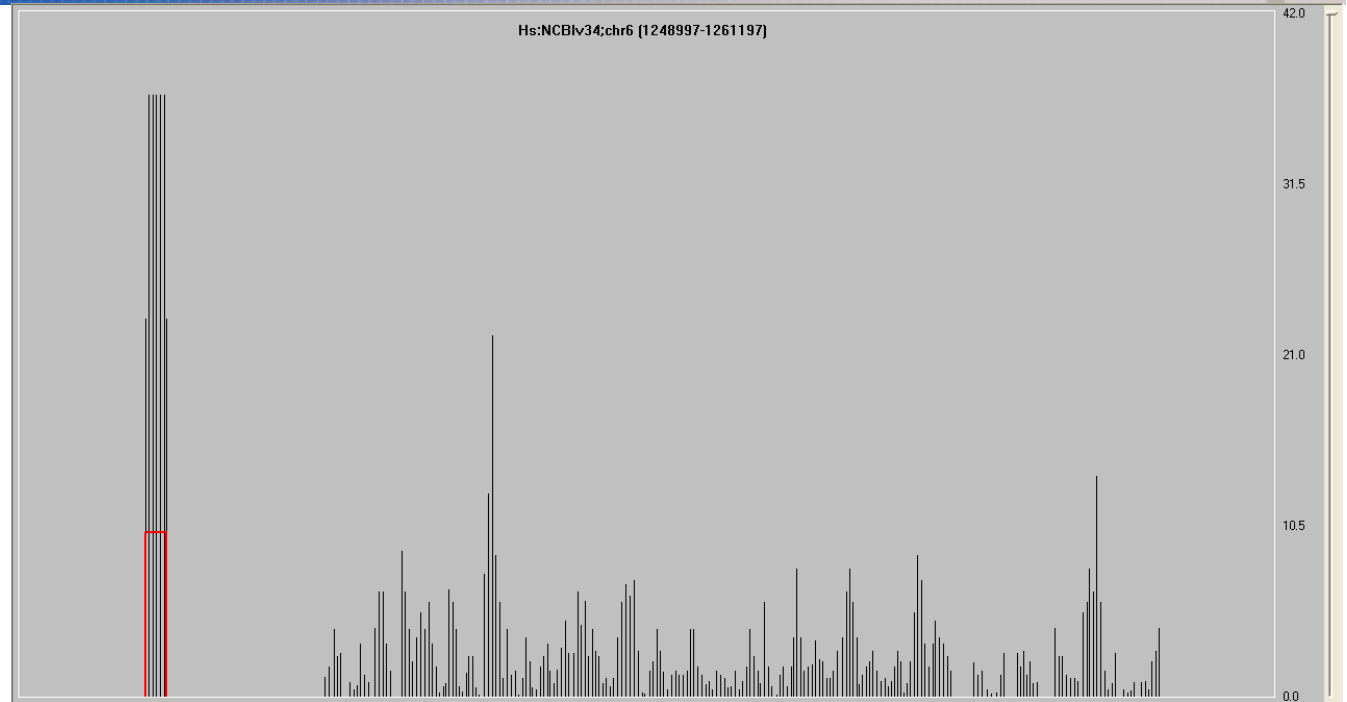
Threshold = 13
Max Gap = 80bp
Min run = 200bp





The Way Ahead.™

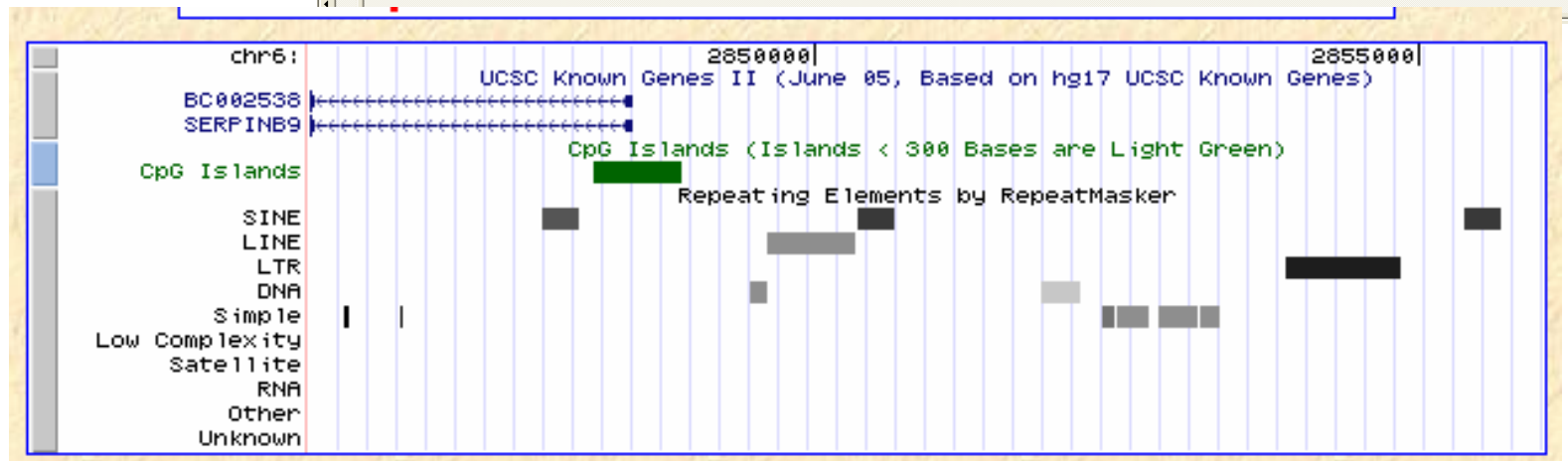
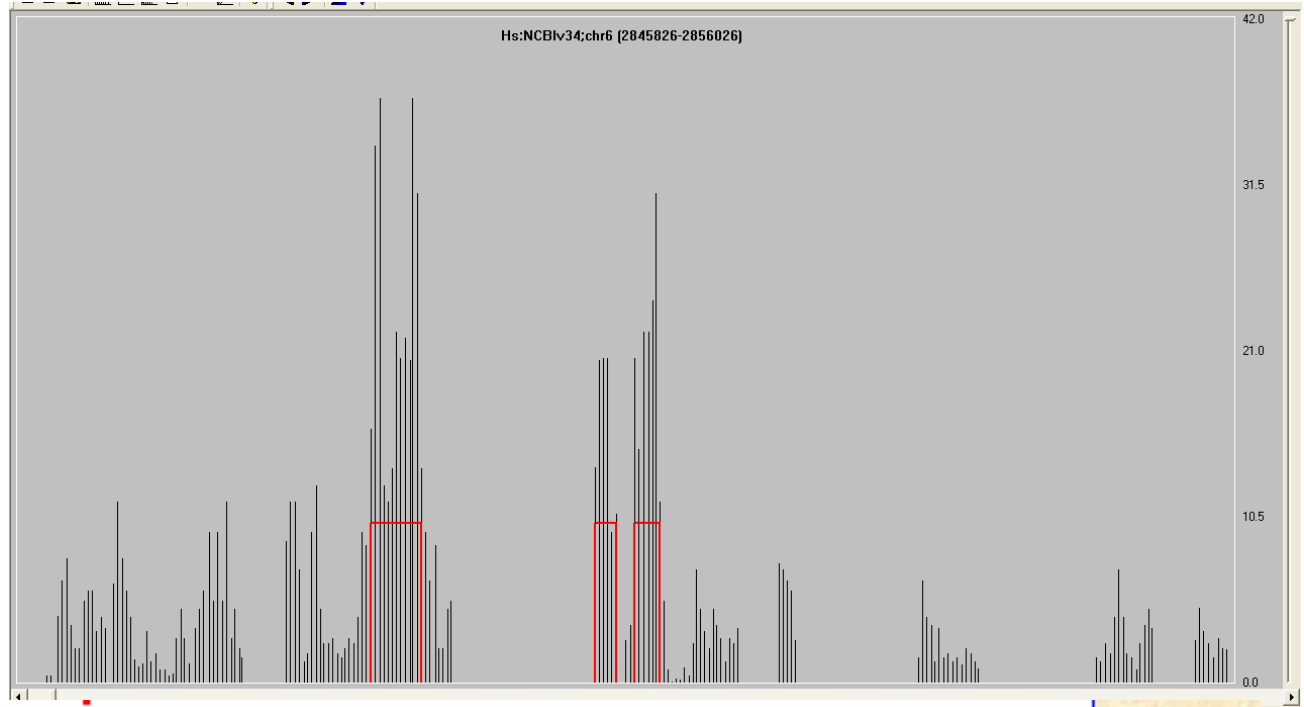
Methylated Repetitive DNA





The Way Ahead.™

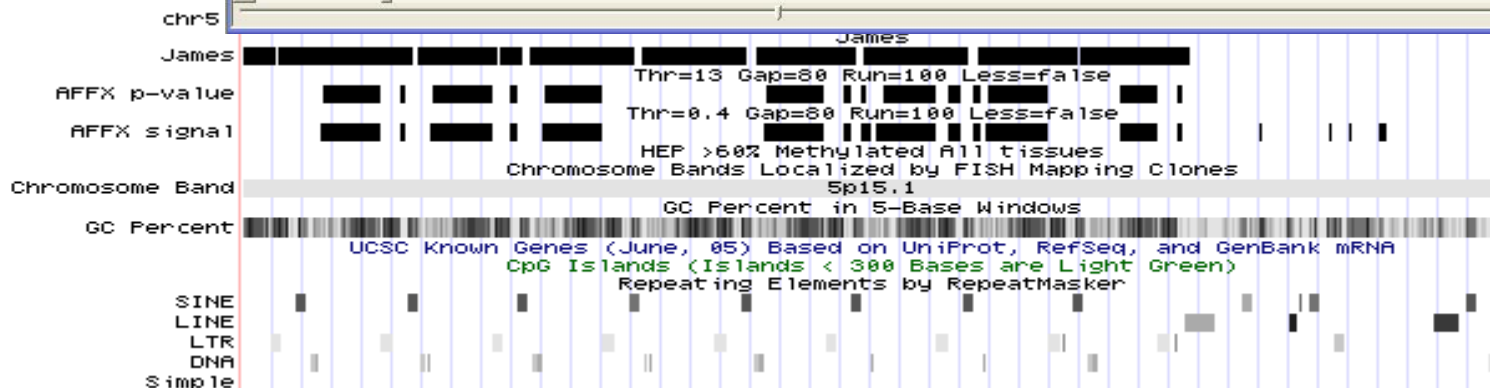
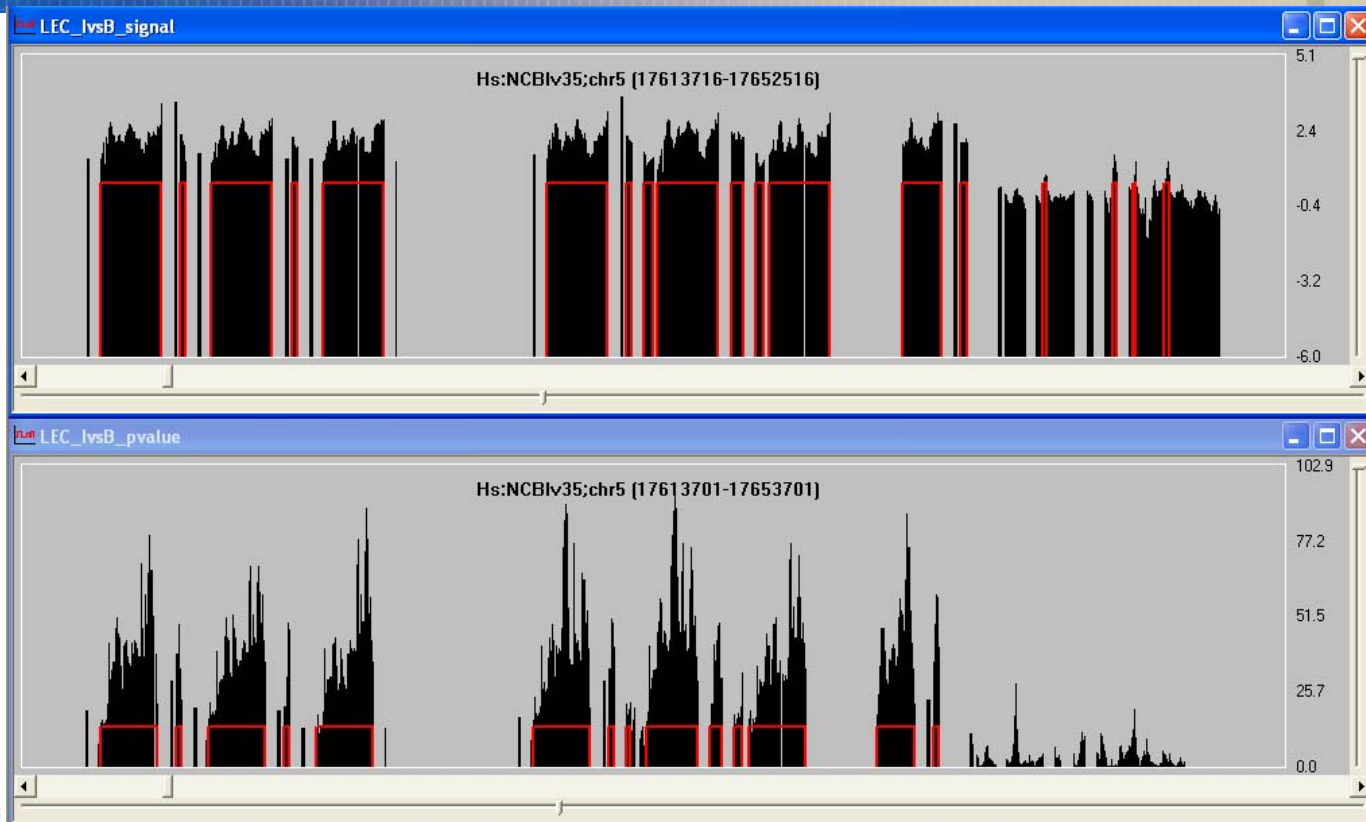
Methylated Repetitive DNA





The Way Ahead.™

Methylated Repetitive DNA



MAT

- Model-based analysis of tiling-arrays for ChIP-chip. Johnson et al *PNAS* 2006
- BandWidth = 200
- MaxGap = 80 ?
- MinProbe = 5 ?
- Var = 1
- Tvalue = 1
- P-value = 1e-5



The Way Ahead.™

Summary - Combined Analysis

	# peaks (all chr)	Overlap with HEP (Chr6,22)	# CGI	# Repeats
TAS – pvalue	11106	31/833	80/833	154/833 18%
TAS - signal	34685	142/2561	471/2561	497/2561 19%
MAT	1726	5/103	2/103	47/103 46%
HEP (chr6,chr22)	1058	132 on array, >80%		

Acknowledgments



j.flanagan@ucl.ac.uk

- CRUK Viral Oncology Laboratory WIBR
 - Chris Boshoff
 - Stephen Henderson
 - Laurence Wild
- Scientific Support Services WIBR
 - Jake Raby
 - Helen Roumeliotou
- Funding

