

Order of activities	HELP Assay
<b>Activity 1</b>	Run agarose gel of MspI and HpaII-digested genomic DNA (Practical Class 1) and ligation-mediated PCR reaction (Practical Class 2)
<b>Activity 2</b>	Discuss results and incorporate Next Generation DNA sequencing using the online Integrated Genome Viewer (IGV)
<b>Activity 3</b>	Discussion of laboratory report



# Next Generation DNA Sequencing (Illumina)

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## The BED File

chromosome

signal strength

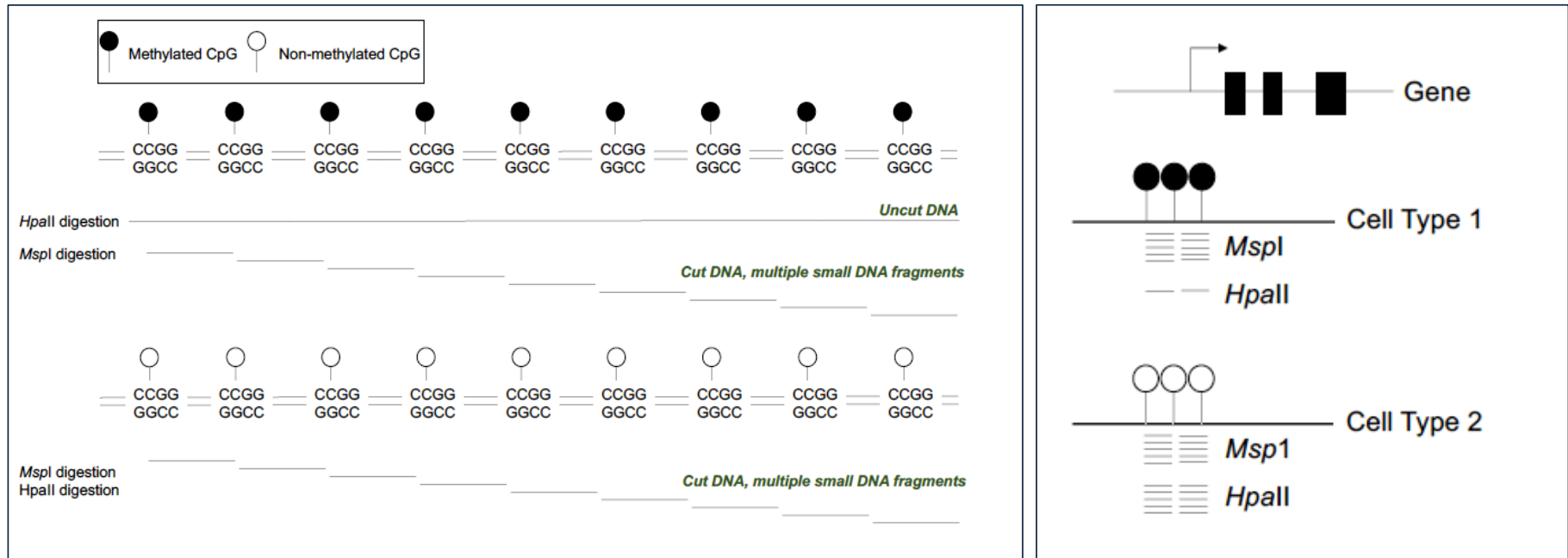
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chr4	128252925	128253101	+	600.8
chr1	156903370	156903546	+	561.7
chr10	90881469	90881645	+	537.8
chr7	87100003	87100179	+	525.3
chr17	29227791	29227967	+	500.5
chr8	23544523	23544699	+	473.0
chr5	140199090	140199266	+	459.7
chr10	117154716	117154892	+	449.0
chr1	54901247	54901423	+	444.6
chr8	64780293	64780469	+	437.5
chr15	85690303	85690479	+	428.6
chr9	117068448	117068624	+	423.3
chr3	32263187	32263363	+	419.7
chr8	23545199	23545375	+	419.7
chr10	117147028	117147204	+	418.8
chr2	167389561	167389737	+	407.3



The genomic coordinates

DNA strand

The HELP assay: analysing DNA methylation by measuring the abundance of small *HpaII* and *MspI* fragments across the genome



**Constructing a Library for Next-Generation DNA:  
the H*p*aII tiny fragment Enrichment by Ligation-mediated PCR (HELP) assay**

CCGG [blue bar] CCGG  
GGCC [blue bar] GGCC

Cut *MspI* or *HpaII*

CGG [blue bar] C  
C [blue bar] GGC

Ligation

Sequencing adaptor CCGG [blue bar] CCGG Sequencing adaptor  
GGCC [blue bar] GGCC

Primer  
→

PCR

Sequencing adaptor CCGG [blue bar] CCGG Sequencing adaptor  
GGCC [blue bar] GGCC

←

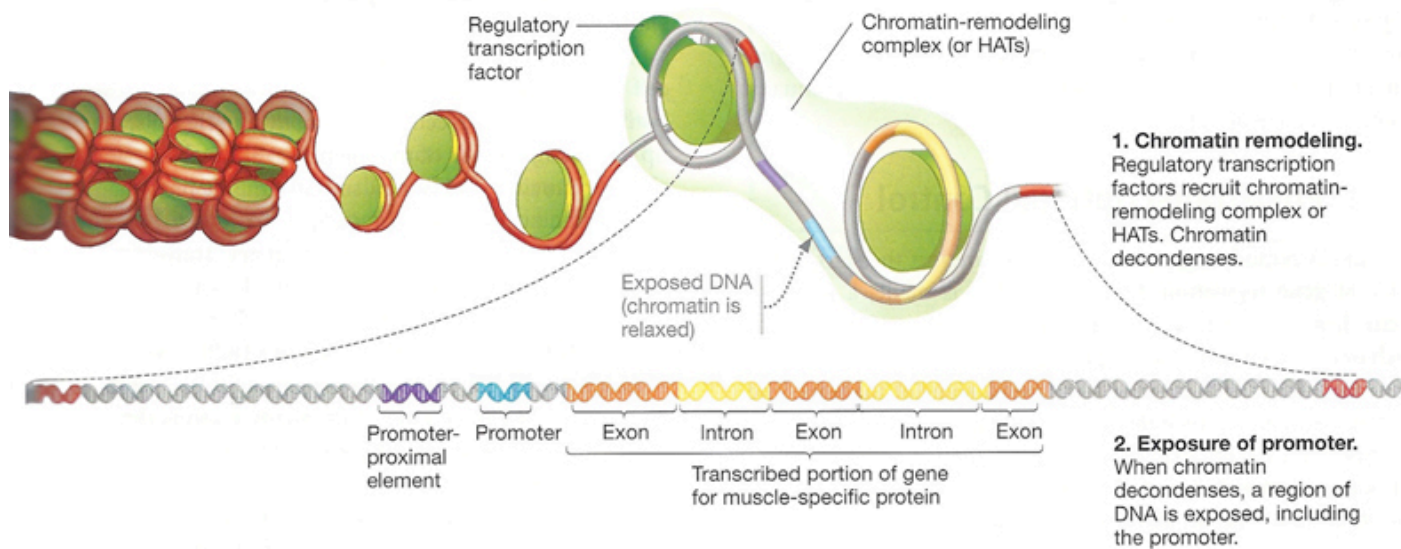
Primer



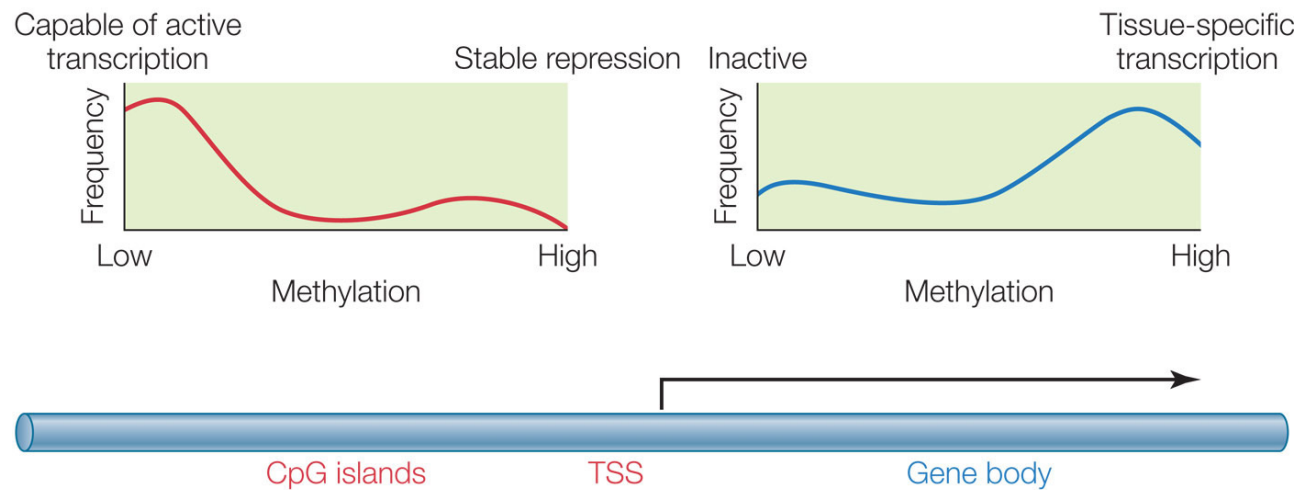
Not cut with *Hpa*II, sequencing adaptor cannot be ligated

- *CpG islands are regions of the genomes rich in CG dinucleotides*
- *CpG islands are typically important for transcription regulation*

Access of transcription factors to a promoter or an enhancer is regulated by dynamic changes in chromatin organization



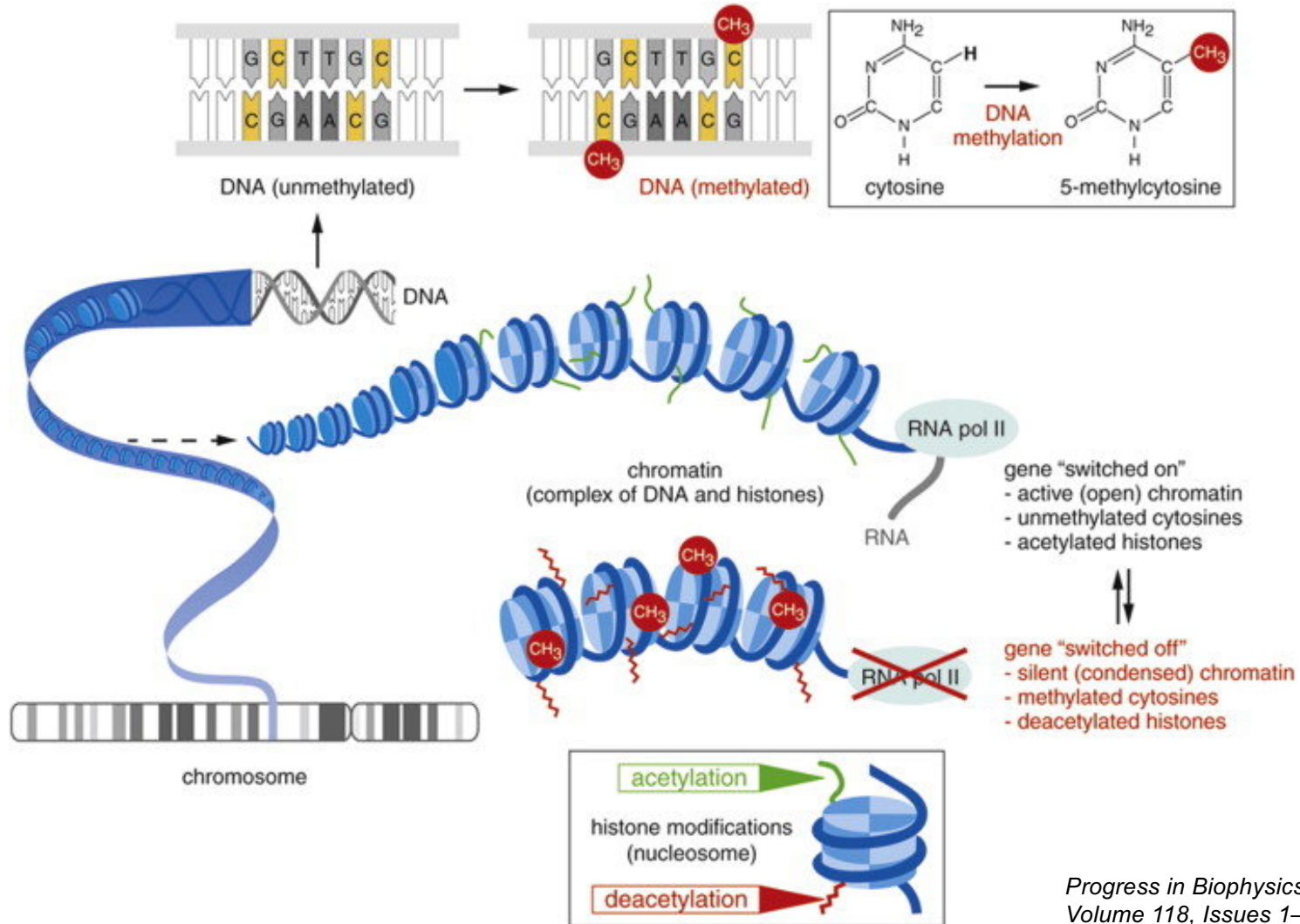
## Methylation and gene transcription



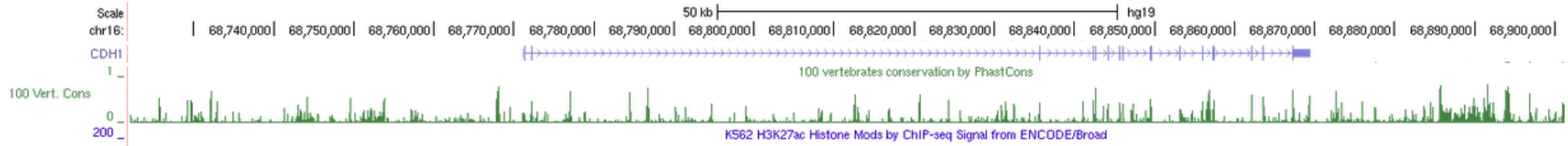
*A PRIMER OF HUMAN GENETICS*, Figure 9.4  
© 2015 Sinauer Associates, Inc.

- *The majority of methylation occurs on the cytosine of 5'-CpG-3' dinucleotides*
- *CpG dinucleotides can be highly enriched at or near promoters, and in these cases are termed CpG islands.*

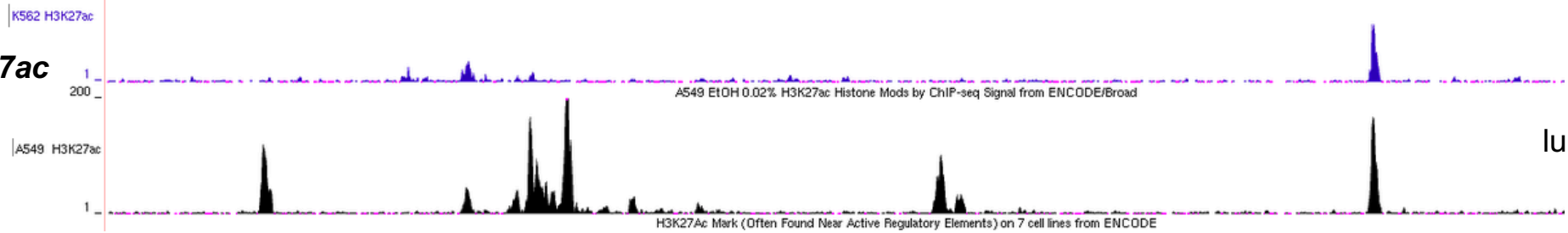
# A link between DNA methylation, histone acetylation, and gene transcription



# CDH1



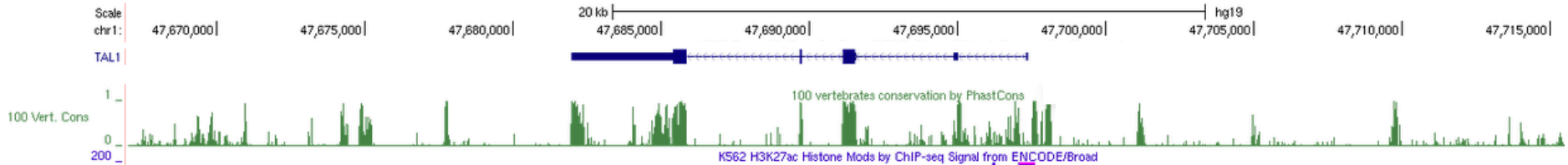
# H3K27ac



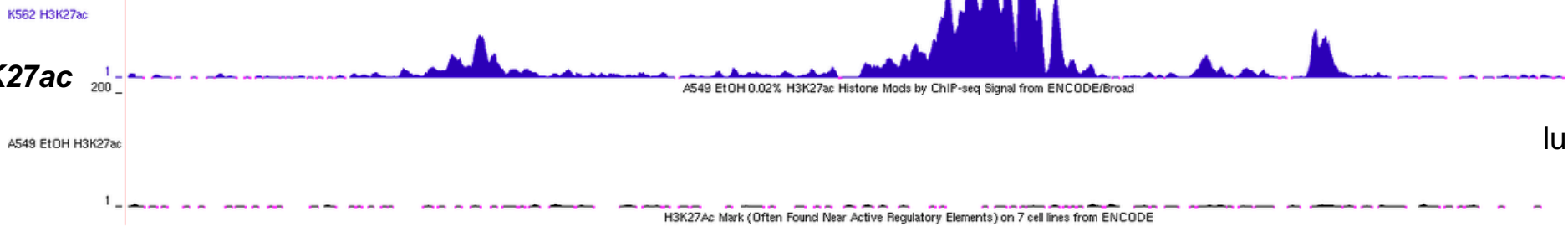
leukemia

lung cancer

# TAL1

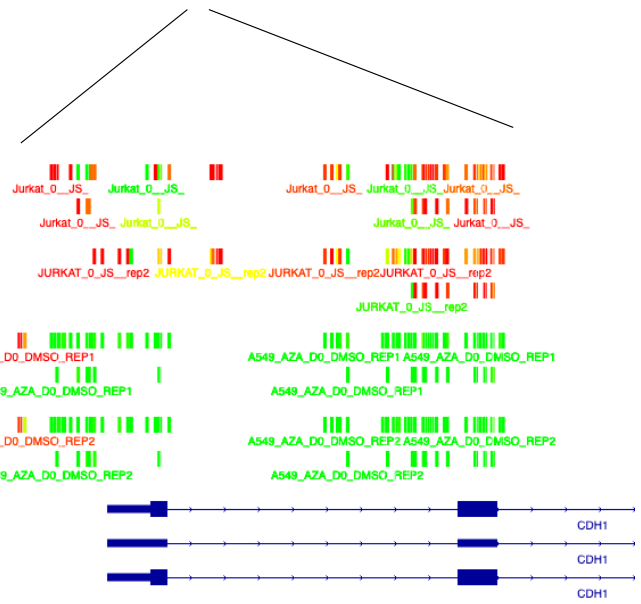
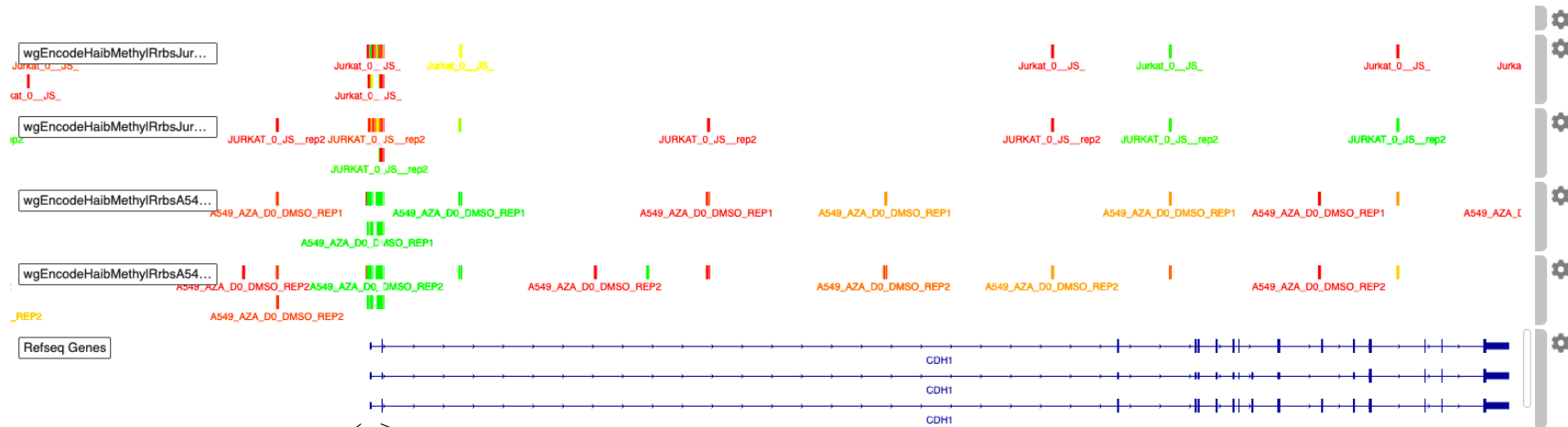


# H3K27ac



leukemia

lung cancer



100% methylated  
 50% methylated  
 0% methylated



wgEncodeHaibMethylRrbsJur...



wgEncodeHaibMethylRrbsJur...



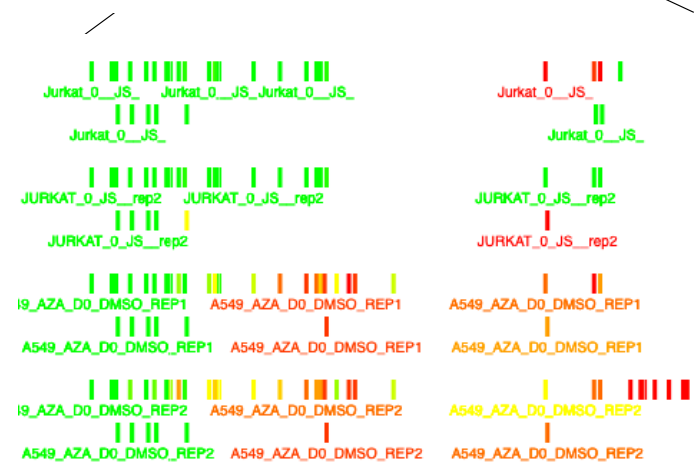
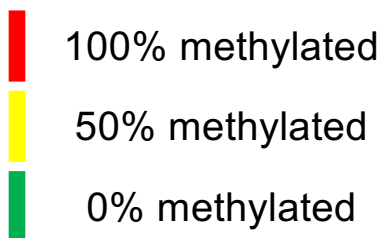
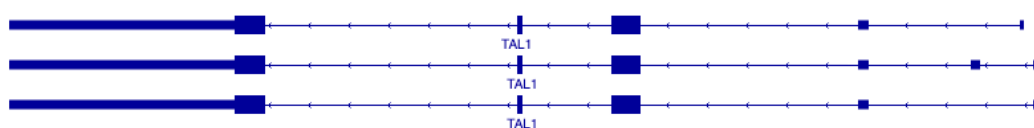
wgEncodeHaibMethylRrbsA54...



wgEncodeHaibMethylRrbsA54...



Refseq Genes



<https://software.broadinstitute.org/software/igv/>

<https://igv.org/app/>

Load Next Generation Sequencing DNA Methylation Files

red = 100% of molecules sequenced are methylated

yellow = 50% of molecules sequenced are methylated

green = 0% of molecules sequenced are methylated

TAL1: chr1:47,680,961-47,705,007

CDH1: chr16:68,755,194-68,875,440

## Methods

1. TaqMan Assay
2. Prepare the Next Generation DNA Sequencing Library

- No dot points
- No tables
- Text-based description of the methods provides a logical description of the steps of each protocol and it must include detail of reagents (you do not need to list the companies), concentrations, times, temperature

digest

ligate

amplify

analyse

Reaction components	Details	Amount in final reaction
<b>gDNA</b>	200 ng/μl	5μg
<b>HpaII <u>OR</u> MspI *</b>	MspI: 20 units/μl HpaII: 10 units/μl	20 units
<b>Restriction endonuclease buffer</b>	Provided as 10x stock	1x in final reaction
<b>Water</b>	Add to make final reaction volume 50μl	Add to make final reaction volume 50μl
<b>DNA digestion</b>	1hr, 37°C	-

Components	Amount
MspI or HpaII-digested gDNA	200ng
Sequencing Adaptor (5μM)	You will add adaptor to a final concentration of either 0.5 or 1μM
5X Rapid ligation buffer	1X in final reaction
Water to a final volume of 19μl	-
Before adding the T4 DNA ligase, heat the sample to 55°C and cool to 22°C over 1 hour	
T4 DNA ligase 5units/μl	5 units in the final reaction

Components for PCR	Stock concentrations	Amount or final concentration in reaction - HpaII	Amount or final concentration in reaction - MspI
Digested and Ligated Template DNA		20ng	40ng
MyTaq	2X	1X	1X
AdaptorSEQ_FWD	5mM	0.5mM	0.5mM
Nuclease-free water	To a final volume of 25μl		

Step	Temperature (°C)	Time
<b>Initial extension</b>	72	10 min
<b>Initial Denaturation</b>	95	1 min
<b>20 cycles</b>	95	30 secs
	72	3 mins
<b>Final Extension</b>	72	10 mins
<b>Hold</b>	10	-

## Results

### TaqMan

1. Amplification Plot
2. Graph (not column graph) – individual data points with mean and standard deviation
3. Mann-Whitney analysis – EGFR Vs RNaseP

### Ligation-mediated PCR (Library)

1. Gel: HpaII and MspI digestion
2. Gel: Amplification of MspI and HpaII
3. Analysis of methylation using Next Generation DNA Sequencing \_CDH1 and TAL1 in leukemia and lung cancer cells, using the IGV genome browser