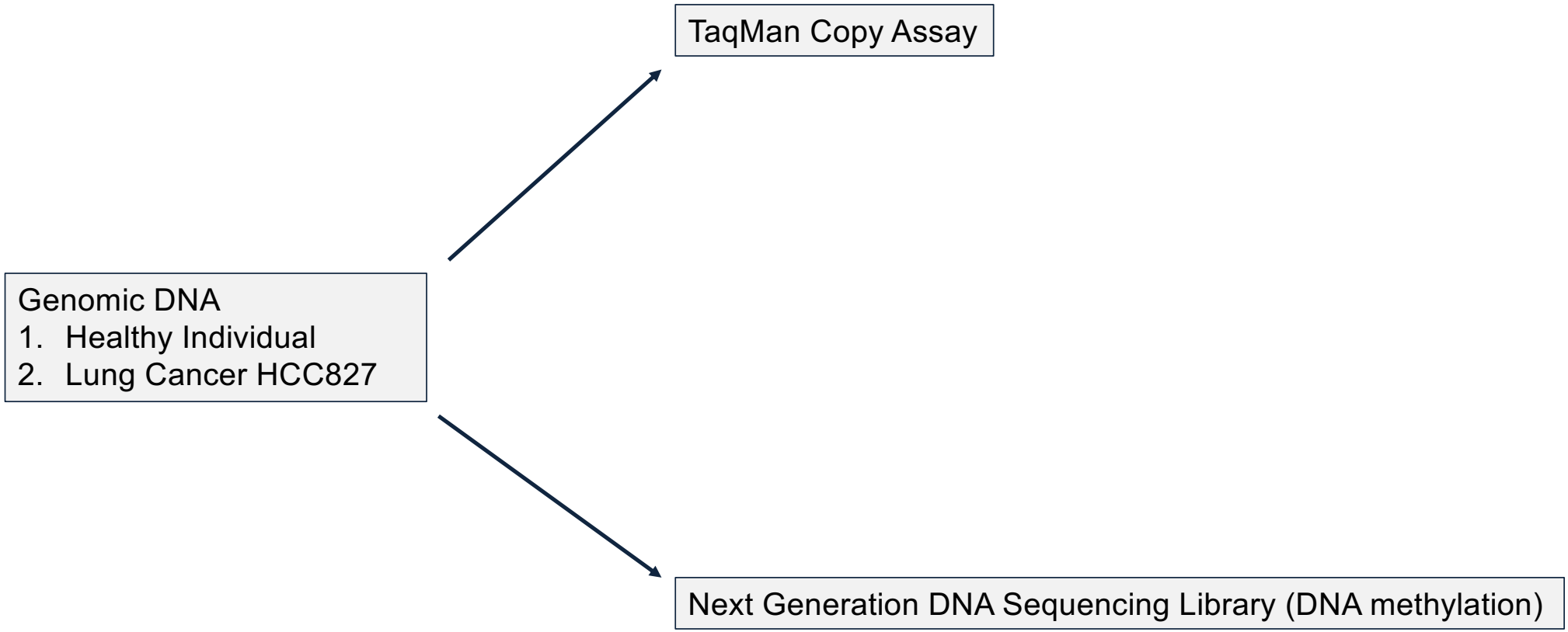
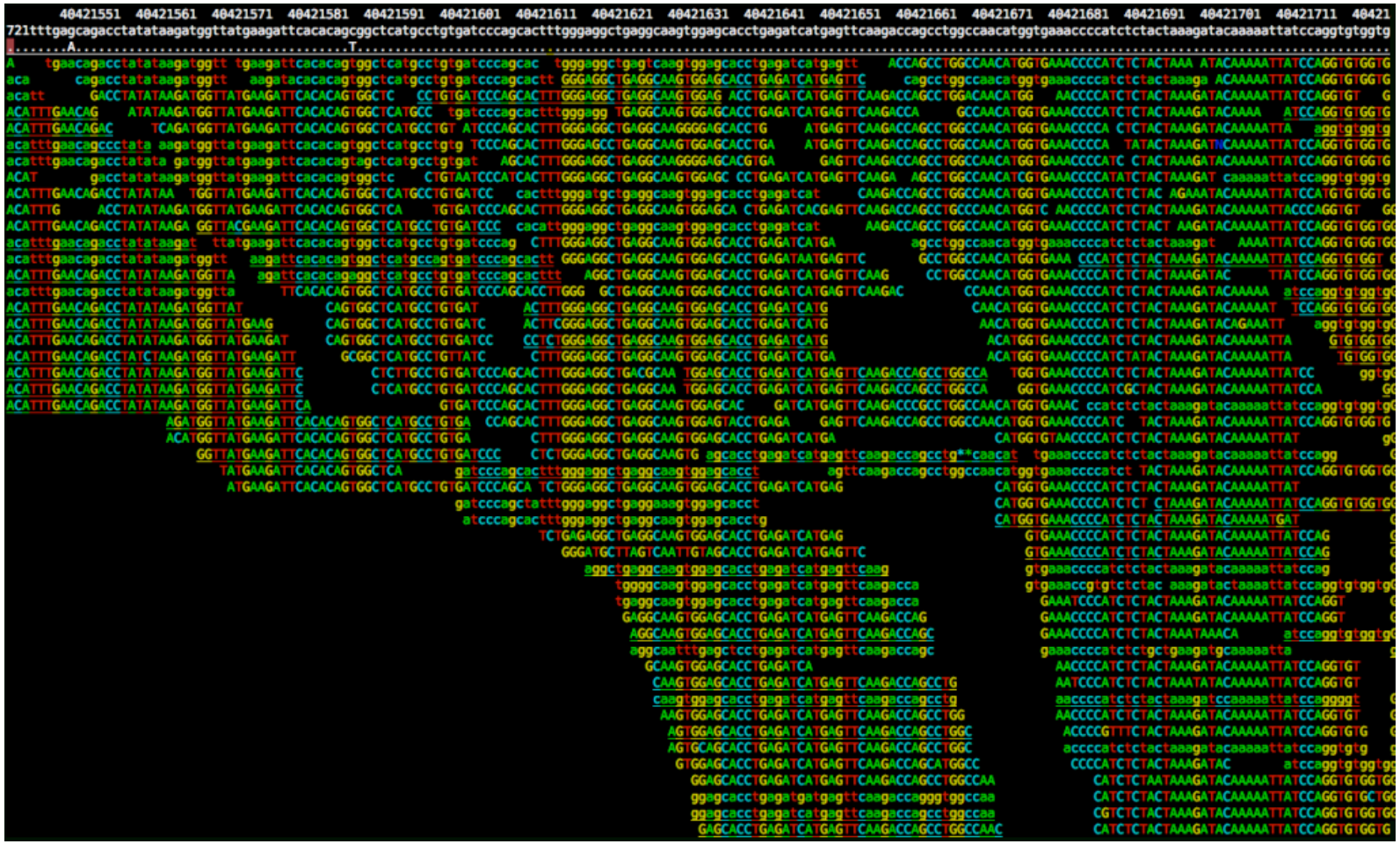

TaqMan Copy Assay
Genomic DNA
1. Healthy Individual
2. Lung Cancer HCC827
Next Generation DNA Sequencing Library (DNA methylation)

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


Raw data
Bioinformatics processing
Conversion to a file that can be read in a genome browser

Next Generation DNA Sequencing (Illumina)



Raw Next Generation DNA Sequencing Output

1. A sequence identifier with information about the sequencing run and the cluster. The exact contents of this line vary by based on the BCL to FASTQ conversion software used.
2. The sequence (the base calls; A, C, T, G and N).
3. A separator, which is simply a plus (+) sign.
4. The base call quality scores. These are Phred +33 encoded, using ASCII characters to represent the numerical quality scores.

Here is an example of a single entry in a R1 FASTQ file:

```
@ML-P2-14:9:000H003HG:1:11102:17290:1073 1:N:0:TCCTGAGC+GCGATCTA
TTTGGTAACAGCATGAATTATTCTAGCCACTAAAACTCTATGAACATCTTGTGAAGGTTTCAGATAGAGCCTGAAGTACACAGAGAACAATTCTTAAAAAA
+
AAAAAEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE<AEEEEEEE
```

The BED File

chromosome

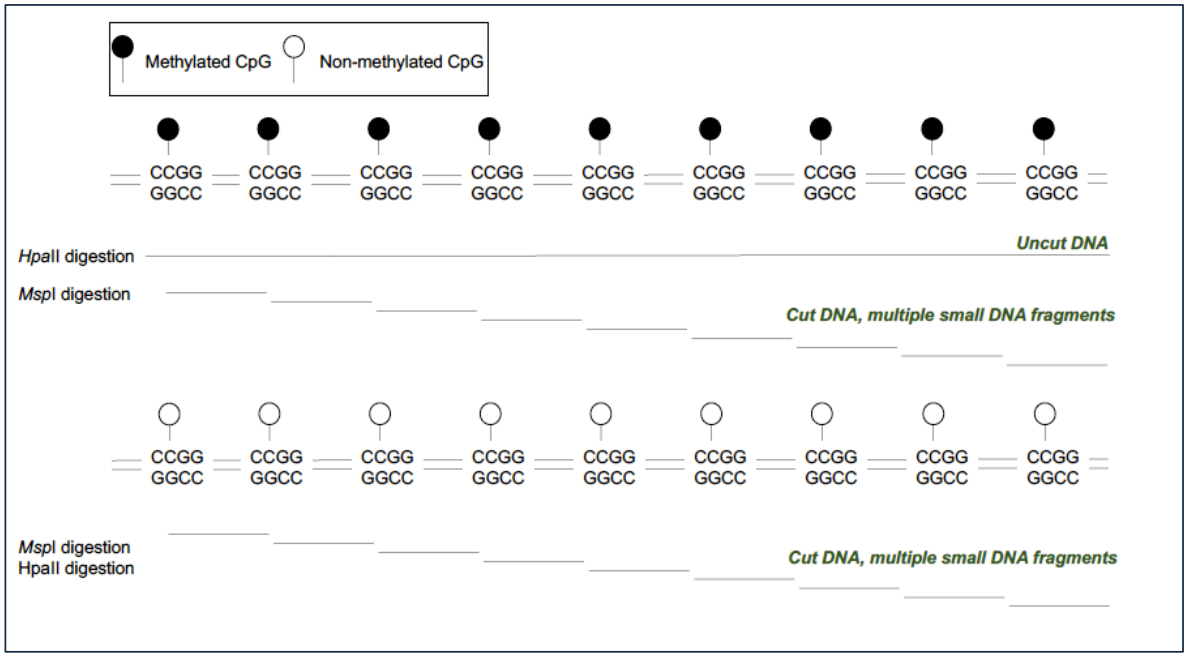
signal strength

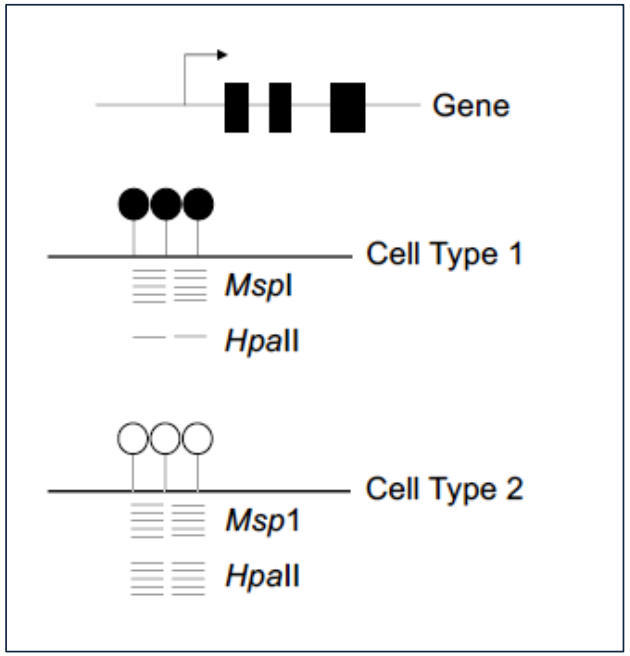
chr8	13548925	13549101	+	1050.7
chr12	111963380	111963556	+	1015.2
chr7	139921178	139921354	+	810.2
chr8	12634989	12635165	+	654.0
chr4	128252925	128253101	+	600.8
chr1	156903370	156903546	+	561.7
chr10	90881469	90881645	+	537.8
chr7	87100003	87100179	+	525.3
chr17	29227791	29227967	+	500.5
chr8	23544523	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

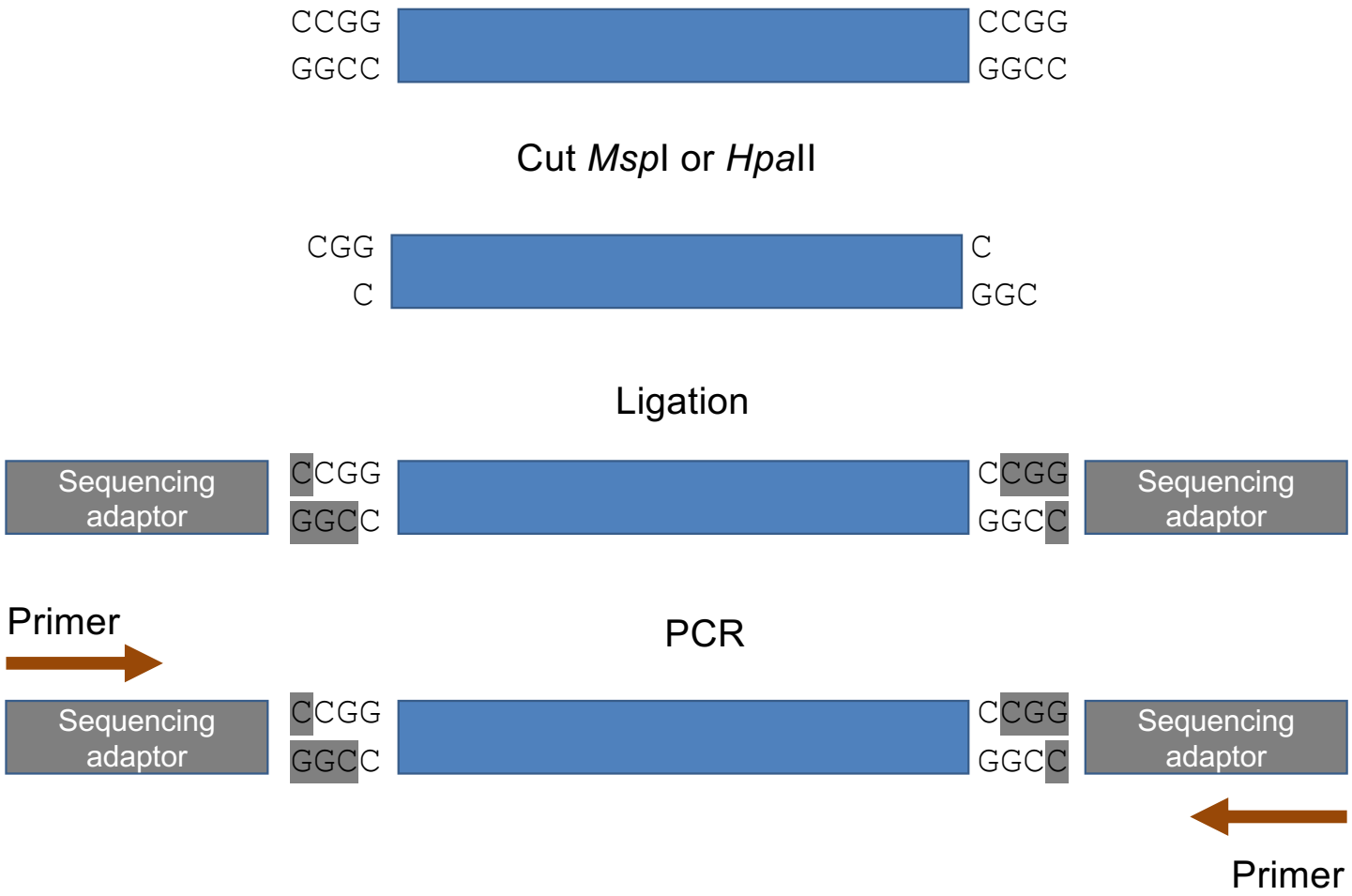
DNA strand

The HELP assay: analysing DNA methylation by measuring the abundance of small *Hpa*II and *Msp*I fragments acrss the genome





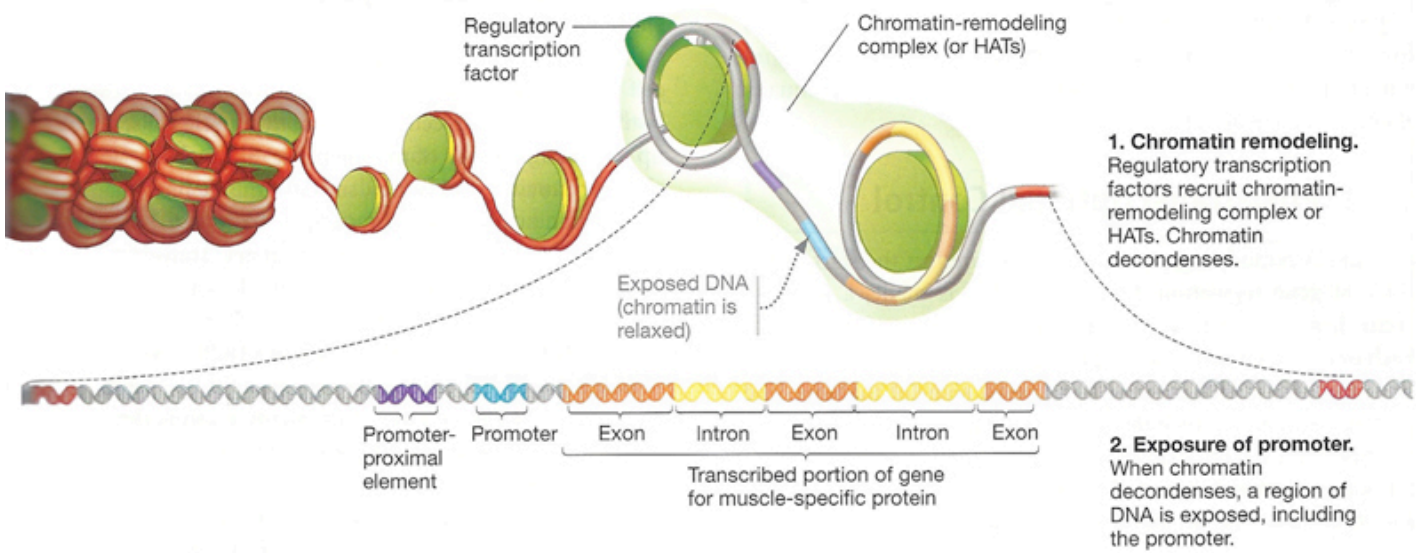
Constructing a Library for Next-Generation DNA:
the *Hpa*II tiny fragment Enrichment by Ligation-mediated PCR (HELP) assay


CCGG
GGCC
CCGG
GGCC
Cut *Msp*I or *Hpa*II
CGG
C
C
GGC
Ligation
Sequencing adaptor
CCGG
GGCC
CCGG
GGCC
Sequencing adaptor
Primer
PCR
Sequencing adaptor
CCGG
GGCC
CCGG
GGCC
Sequencing adaptor
Primer

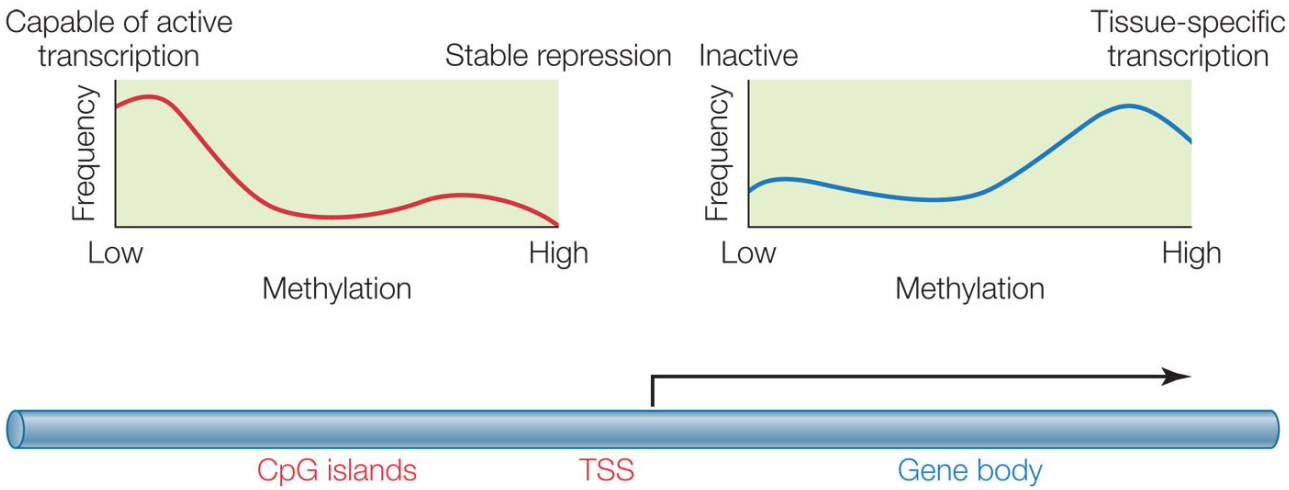
Not cut with *Hpa*II, sequencing adaptor cannot be ligated

- *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



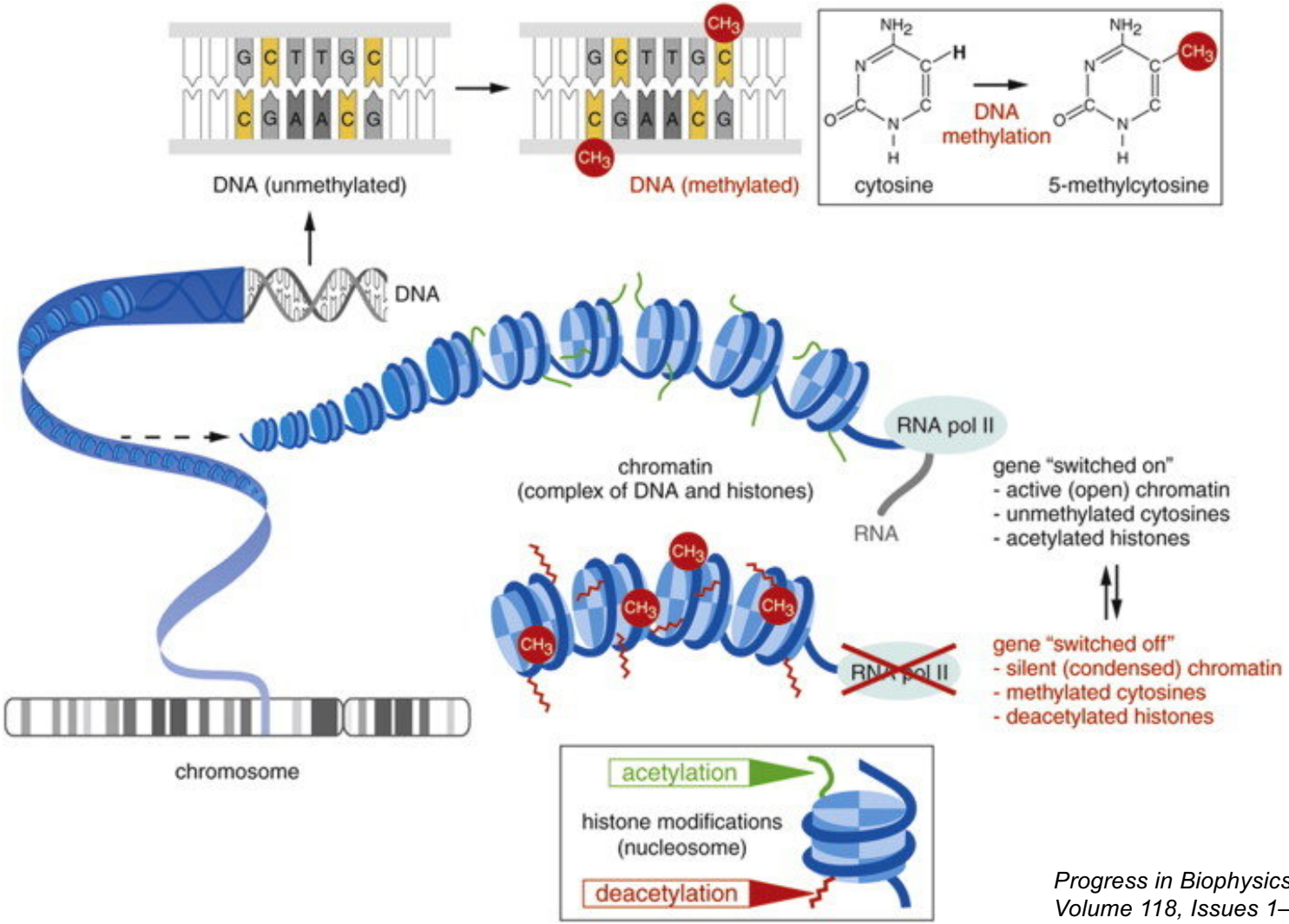
Methylation and gene transcription



***A PRIMER OF HUMAN GENETICS*, Figure 9.4**

- *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



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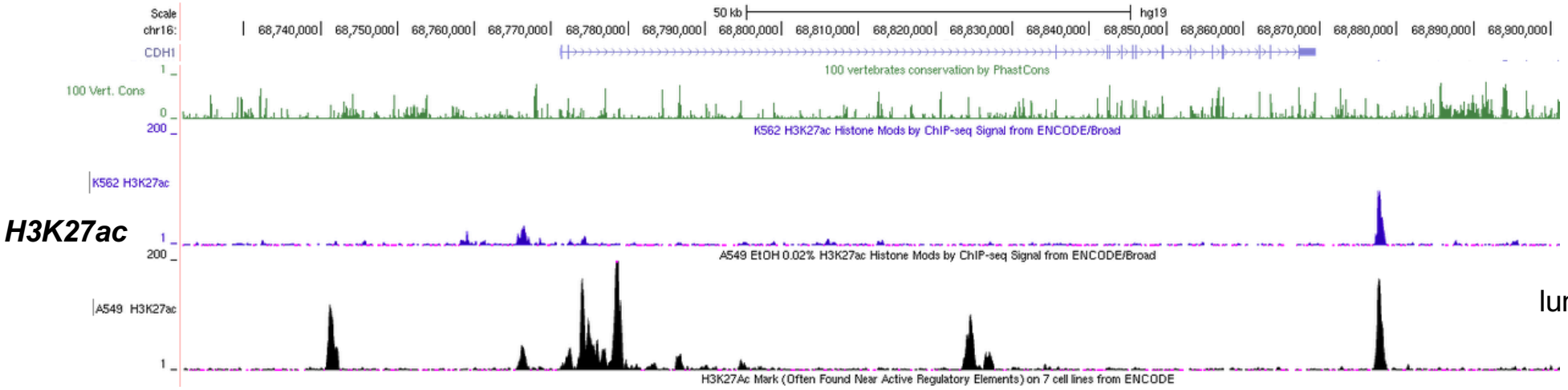
Volume 118, Issues 1–2, July 2015, Pages 69-78

CDH1

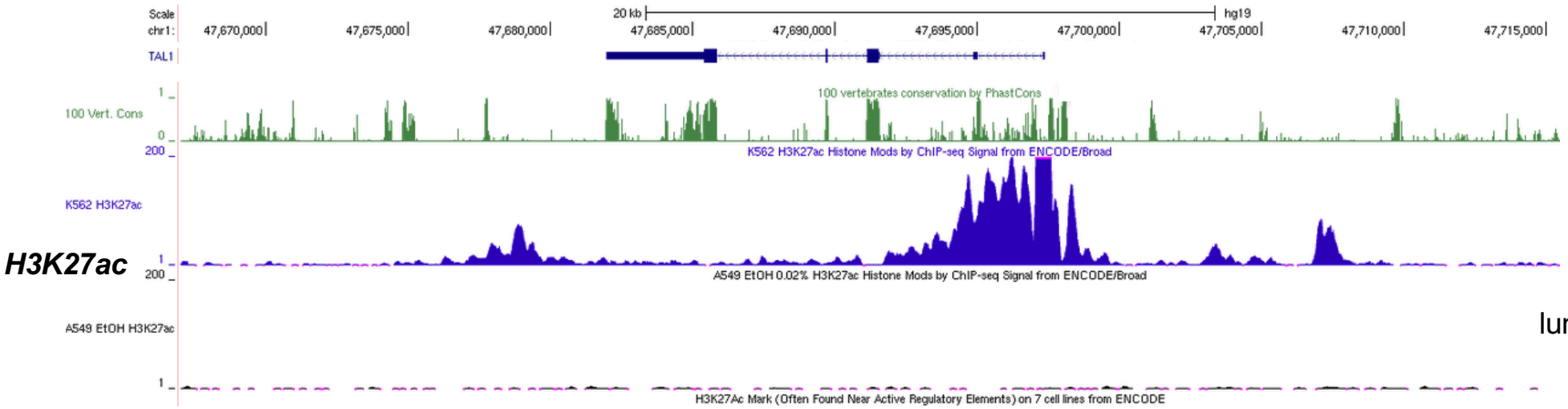


leukemia

H3K27ac

lung cancer

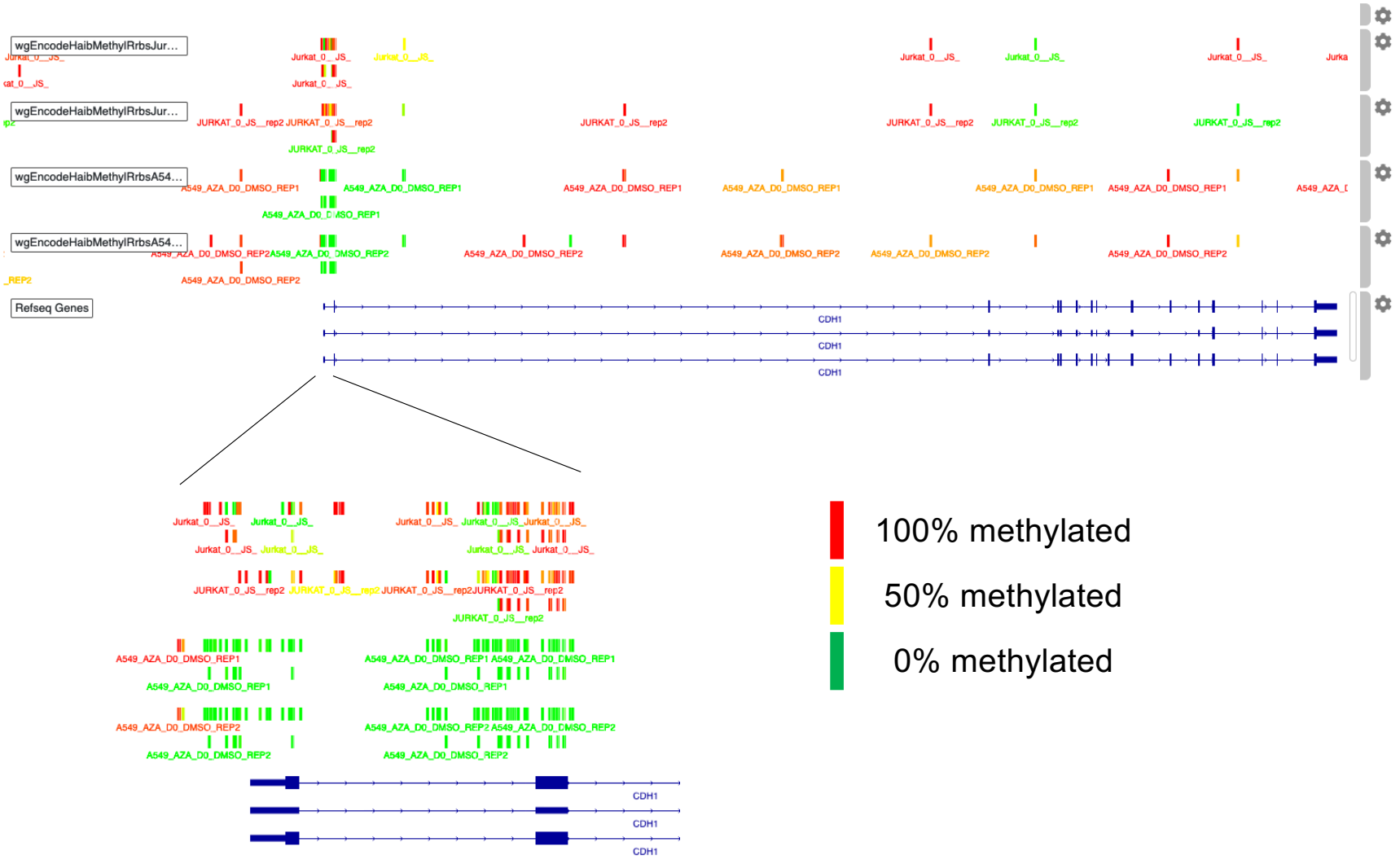
TAL1

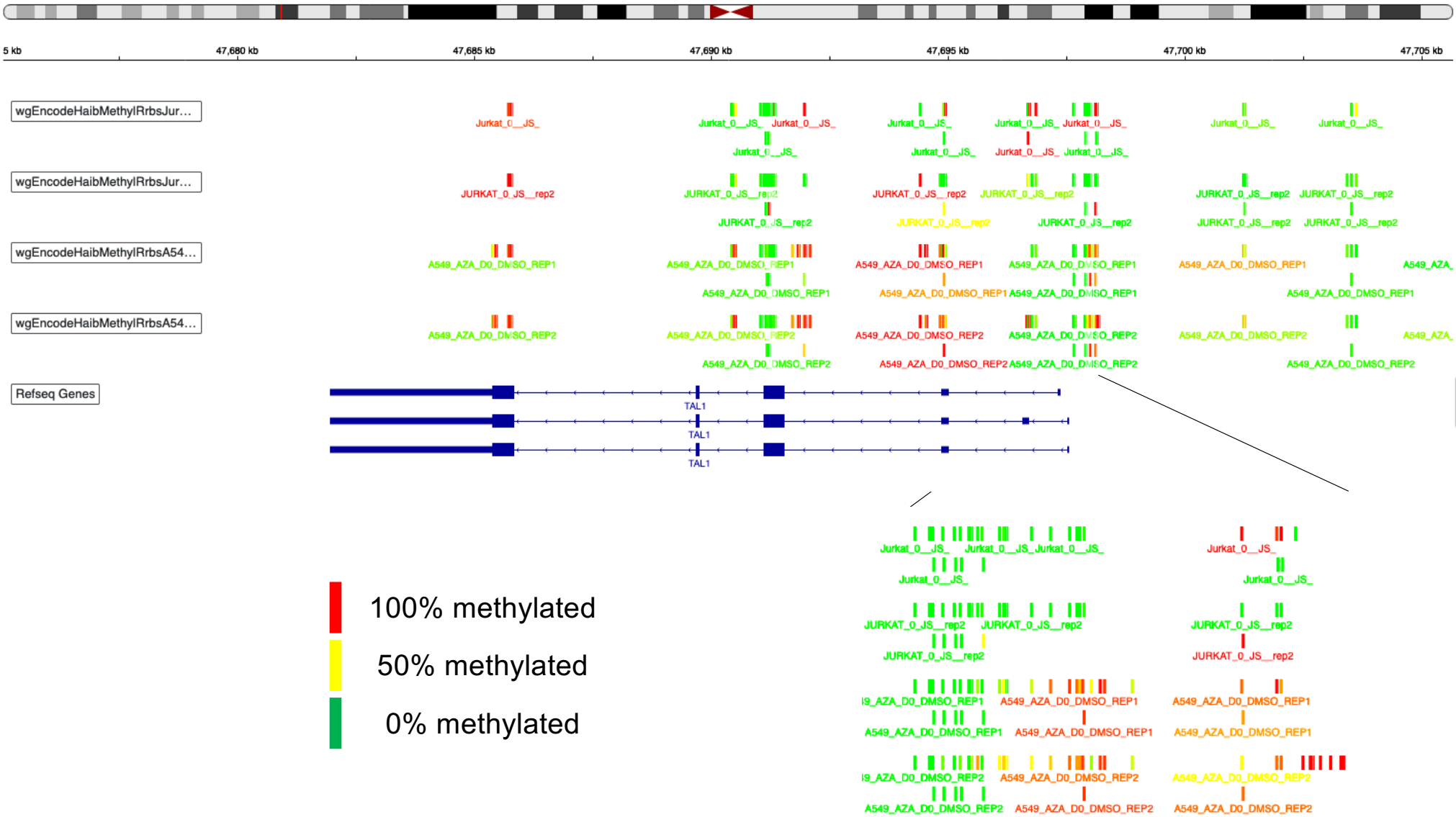


leukemia

H3K27ac

lung cancer


wgEncodeHaibMethylRrbsJur…
wgEncodeHaibMethylRrbsJur…
wgEncodeHaibMethylRrbsA54…
wgEncodeHaibMethylRrbsA54…
Refseq Genes
CDH1
CDH1
CDH1
100% methylated
50% methylated
0% methylated
CDH1
CDH1
CDH1


5 kb
47,680 kb
47,685 kb
47,690 kb
47,695 kb
47,700 kb
47,705 kb
wgEncodeHaibMethylRrbsJur…
wgEncodeHaibMethylRrbsJur…
wgEncodeHaibMethylRrbsA54…
wgEncodeHaibMethylRrbsA54…
Refseq Genes
TAL1
TAL1
TAL1
100% methylated
50% methylated
0% methylated

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

Reaction components	Details	Amount in final reaction
gDNA	200 ng/μl	5μg
HpaII OR MspI *	MspI: 20 units/μl HpaII: 10 units/μl	20 units
Restriction endonuclease buffer	Provided as 10x stock	1x in final reaction
Water	Add to make final reaction volume 50μl	Add to make final reaction volume 50μl
DNA digestion	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
Initial extension	72	10 min
Initial Denaturation	95	1 min
20 cycles	95	30 secs
	72	3 mins
Final Extension	72	10 mins
Hold	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