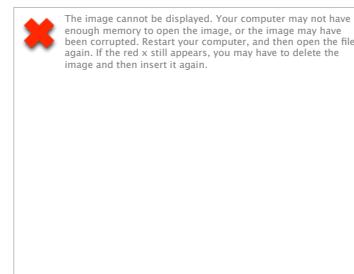
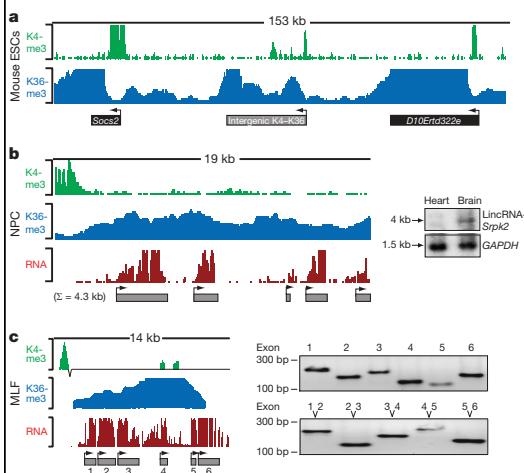


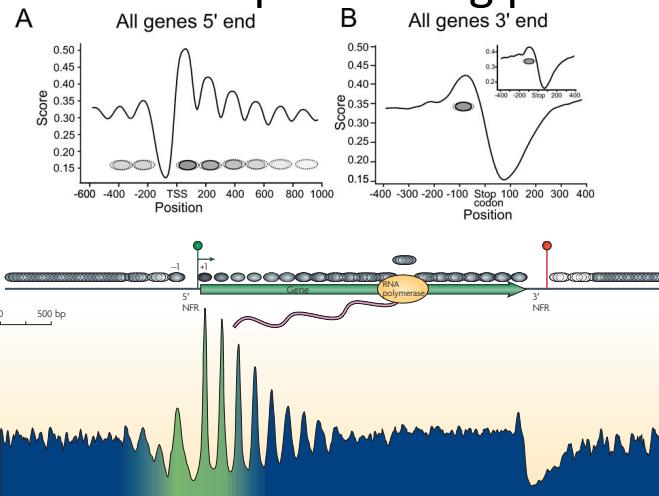
“But DNA isn’t really like that. It’s more like a script. Think of Romeo and Juliet, for example. In 1936 George Cukor directed Leslie Howard and Norma Shearer in a film version. Sixty years later Baz Luhrmann directed Leonardo DiCaprio and Claire Danes in another movie version of this play. Both productions used Shakespeare’s script, yet the two movies are entirely different. Identical starting points, different outcomes.”

-- Nessa Carey, The Epigenetics Revolution

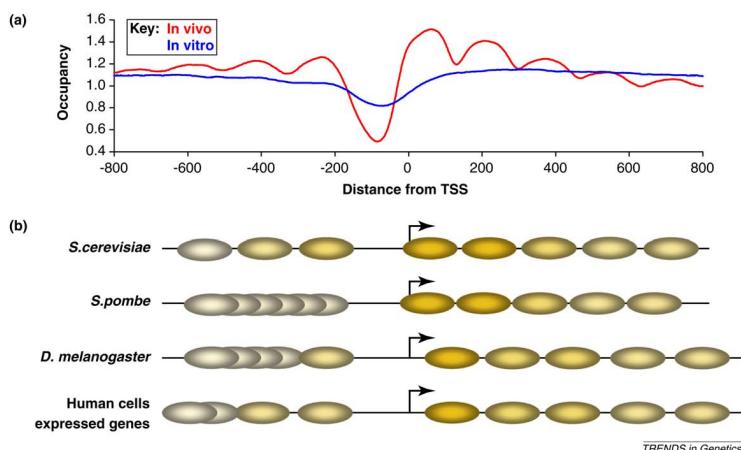
## Finding ncRNA by modification patterns



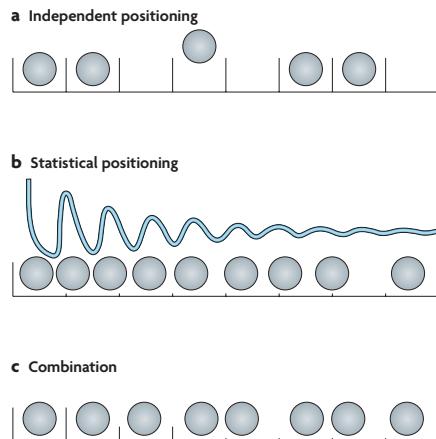
## ChIP for histone protein can identify nucleosome positioning patterns.



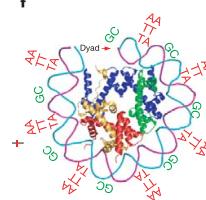
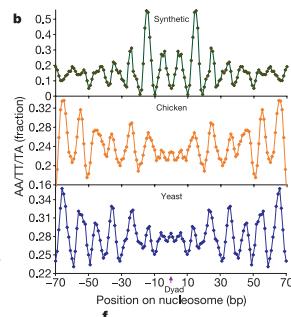
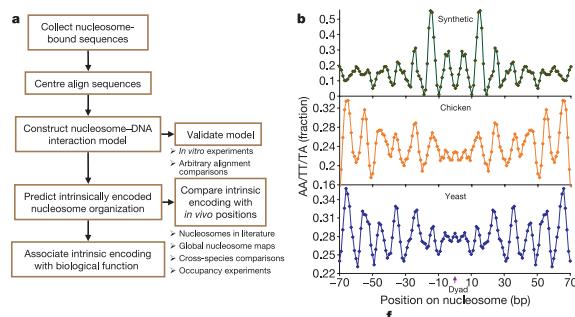
## Positioning is stereotypic across evolution



## Alternative Positioning Theories

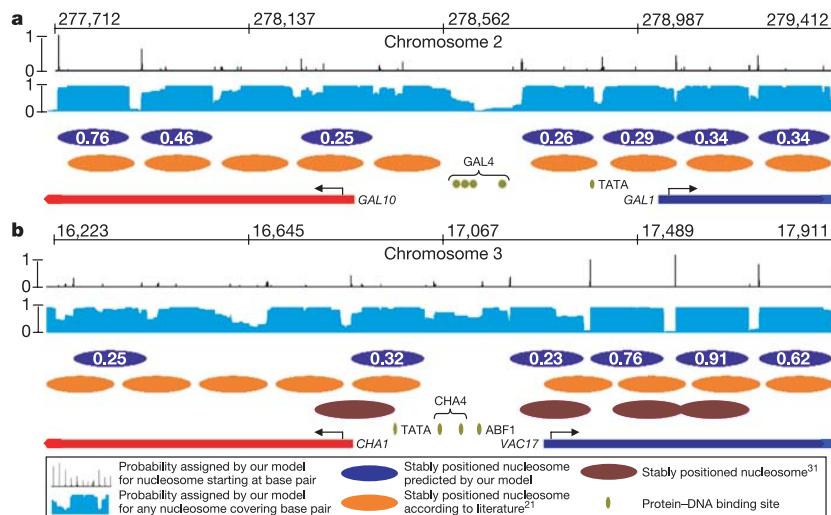


## Computational Models of Nucleosome Positions



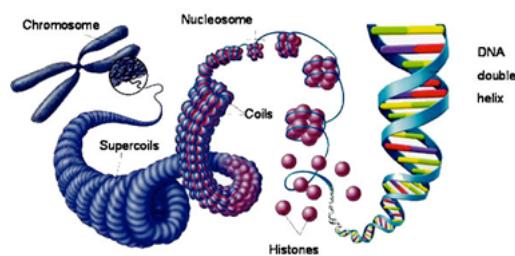
Note that this model is essentially just a fancy position specific scoring system.

## Accuracy of Computational Model

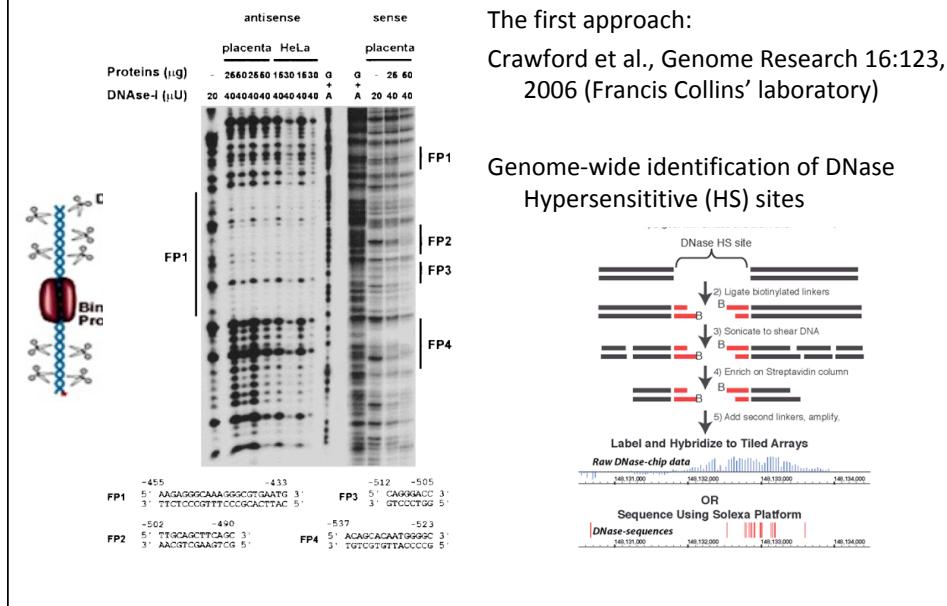


## Ultimately, there are limits on nucleosome positioning by ChIP

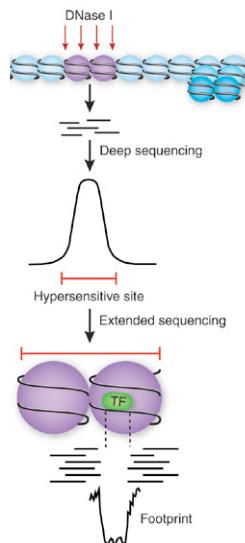
- When ChIP is used on nucleosome core proteins, the “enrichment” possible is highly limited.
- This is particularly true in higher eukaryotes (bigger cells, larger genomes).



The alternative is chromatin accessibility

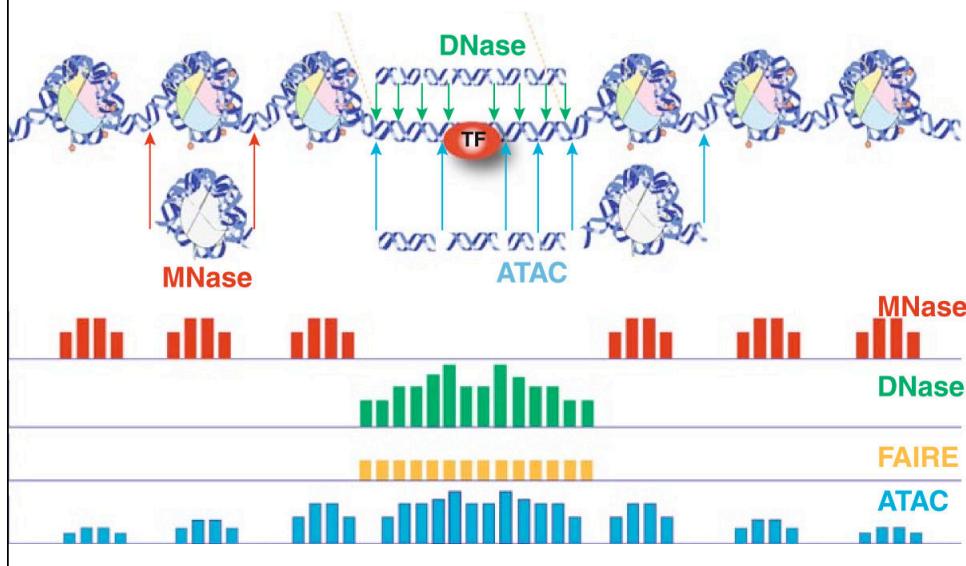


DNAse sensitive regions  
that permits transcription factor “Footprinting”

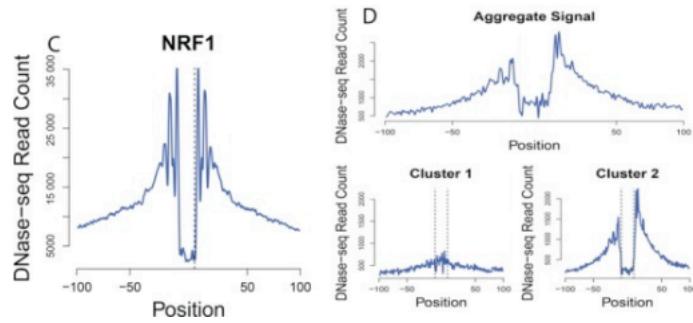


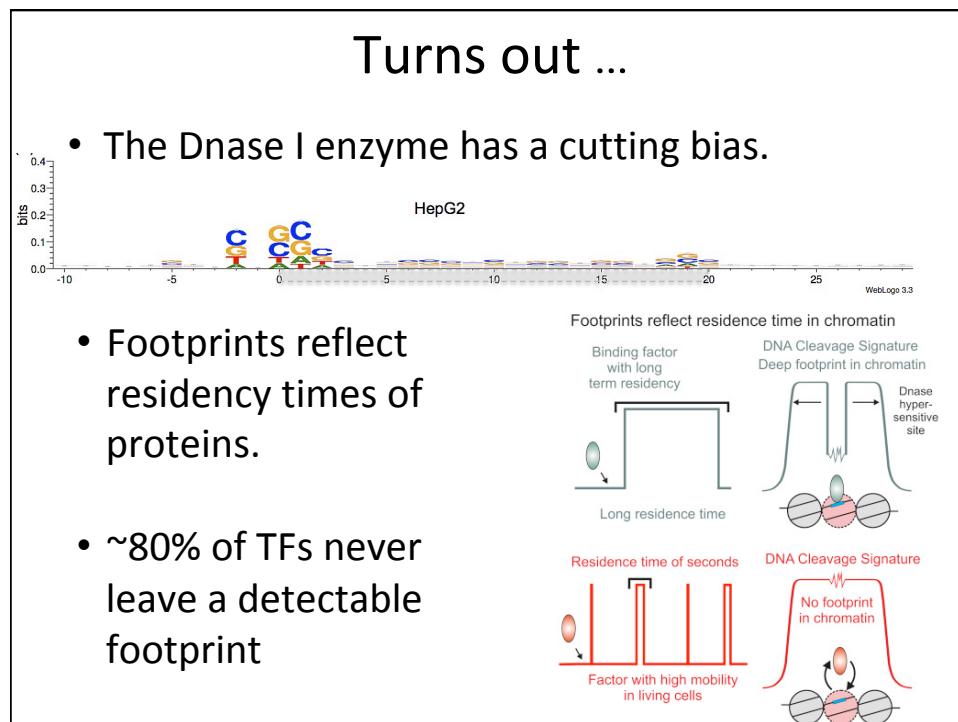
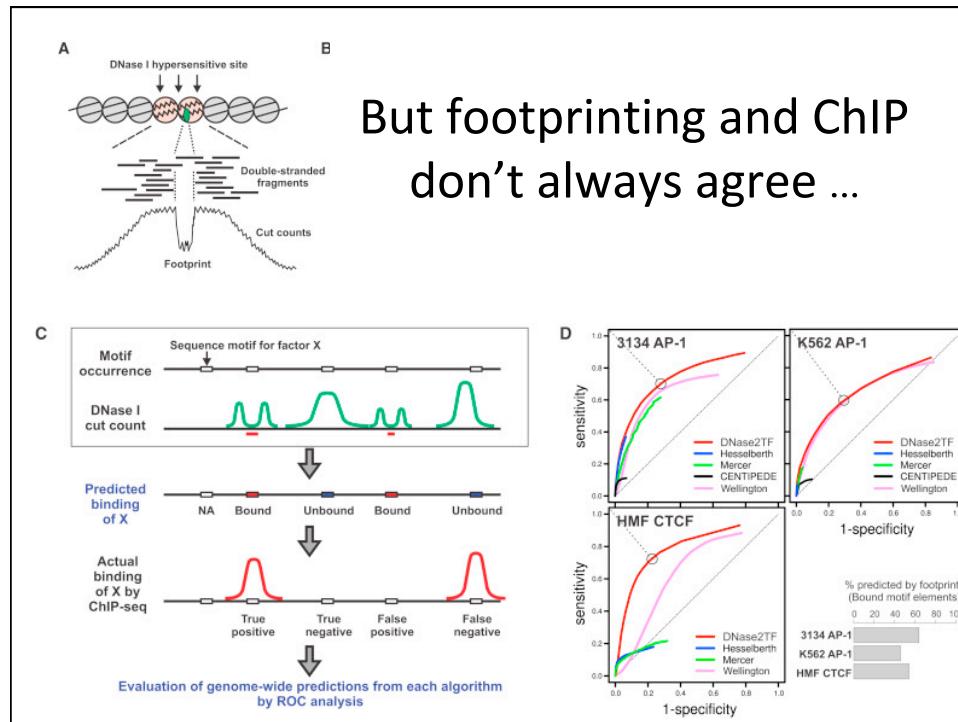
- The easiest method uses low concentrations of DNase I to generate short fragments at sensitive (“open” sites)
  - Permits DNase Footprinting: Very deep sequencing can “see” short protected regions that are absent from the released DNA, and appear as protected “valleys” inside the DNase sensitive peaks
    - protected from DNase I because they are occupied by TF proteins

## Multiple ways to measure accessibility



## Footprinting can identify TF binding sites without antibodies or ChIP

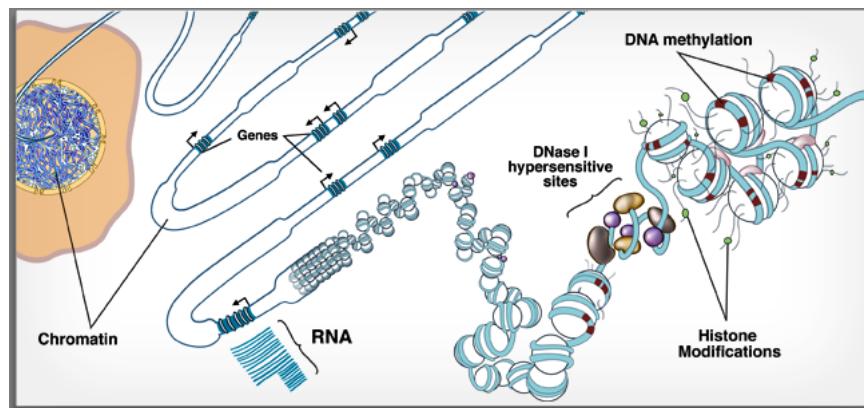




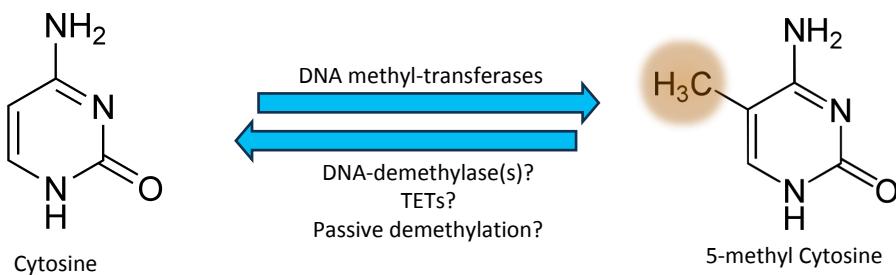
## Epigenetic questions:

- What about even higher order chromatin structure (e.g. 3D packing within nucleus)?  
Hi-C; ChIA-PET, 3C, 4C, 5C
- What about DNA methylation (another epigenetic element)?  
Bisulfide sequencing, enzyme cleavage, m-CIP
- What about RNAs that encode unusual functions?  
Histone marks, RNA-seq, nascent transcription

## Roadmap Epigenomics Project



# DNA Methylation

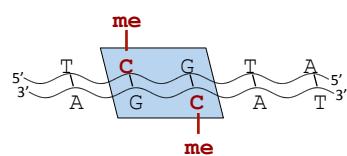


17

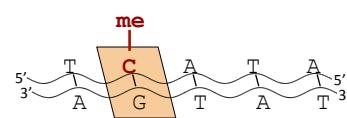
## Types of DNA methylation

	Plants	Mammals
canonical	CG	symmetric

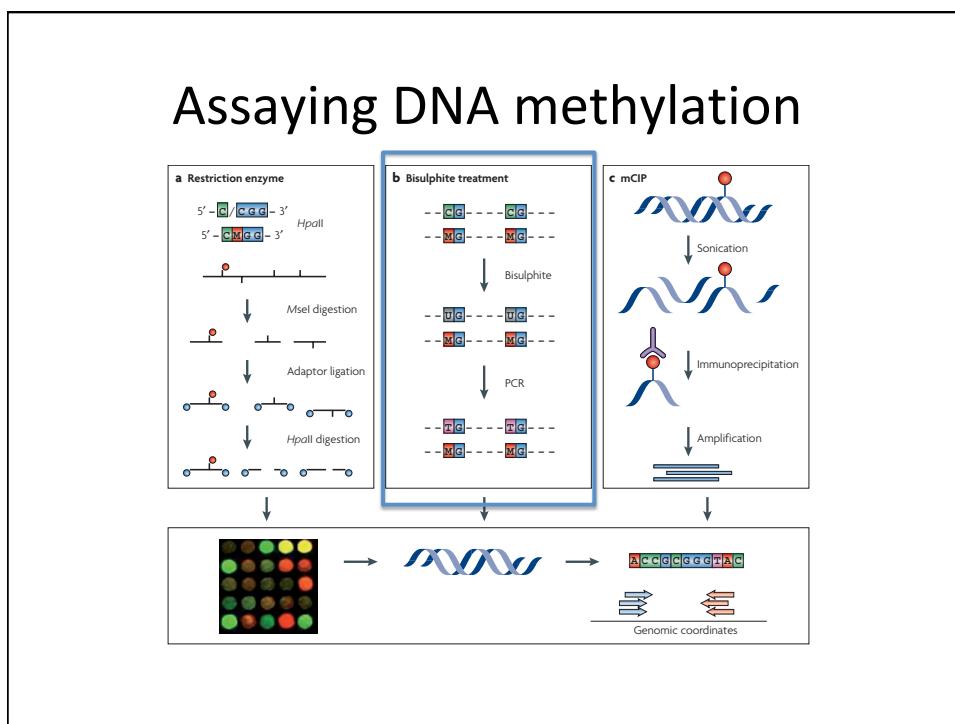
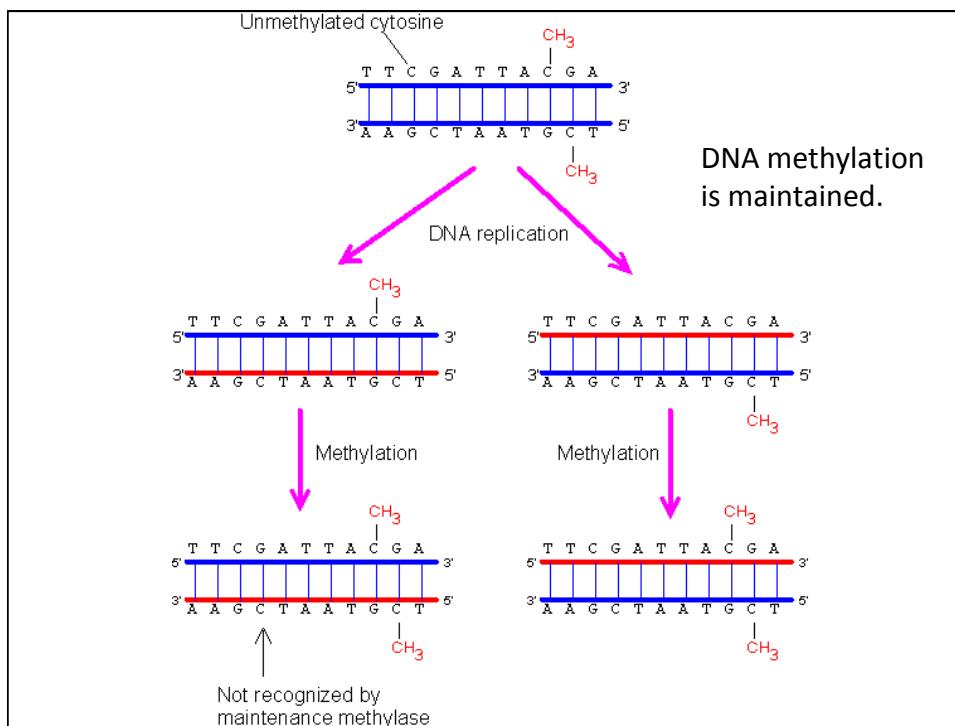
CG context

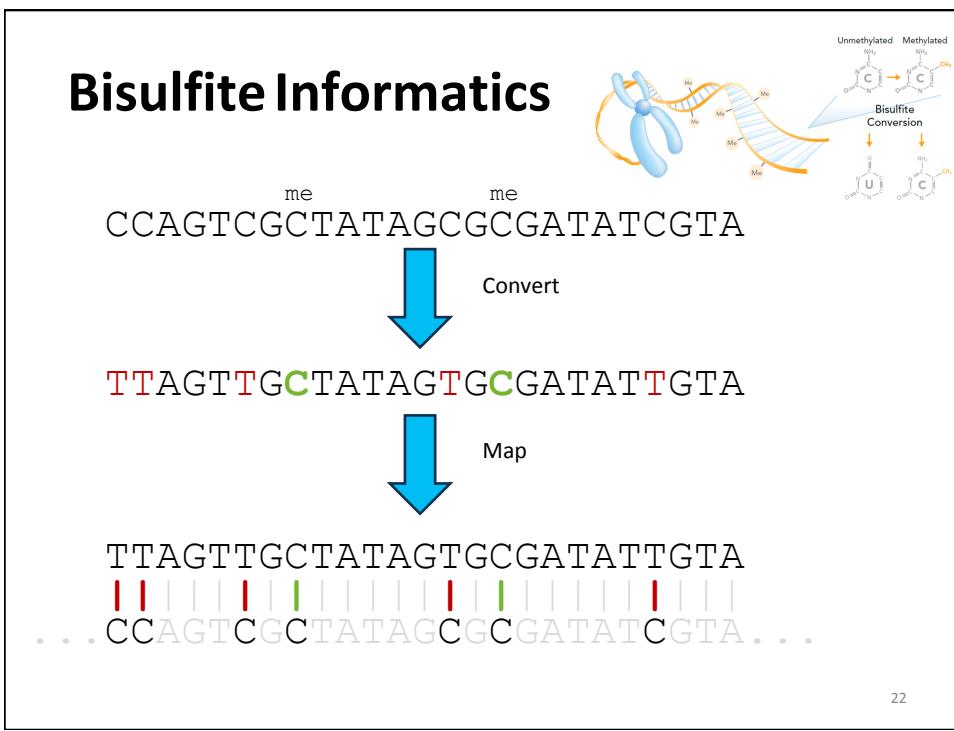
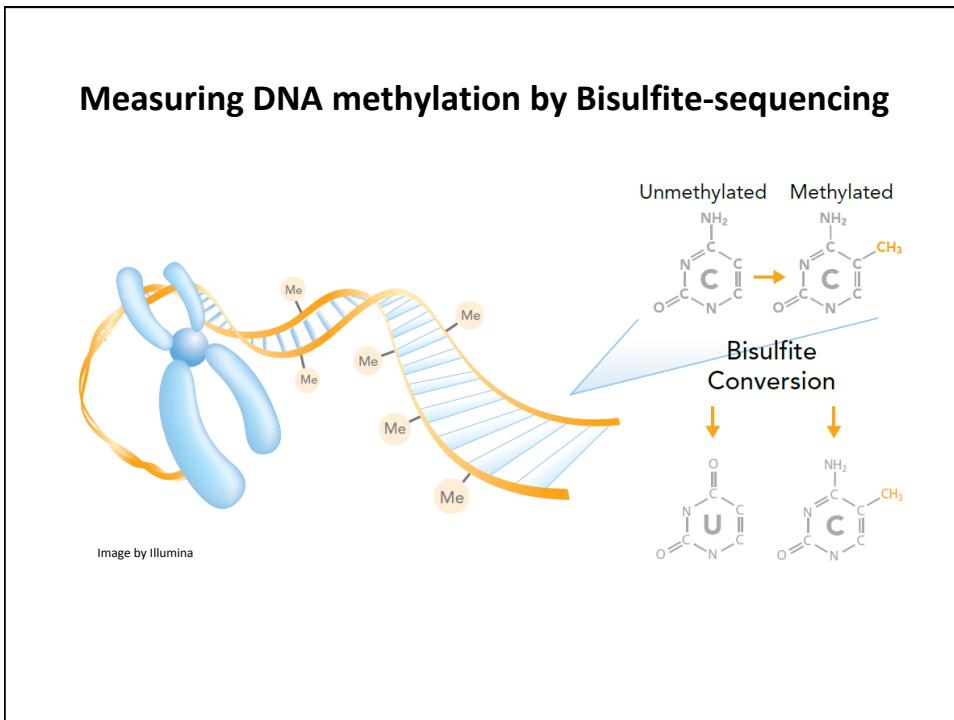


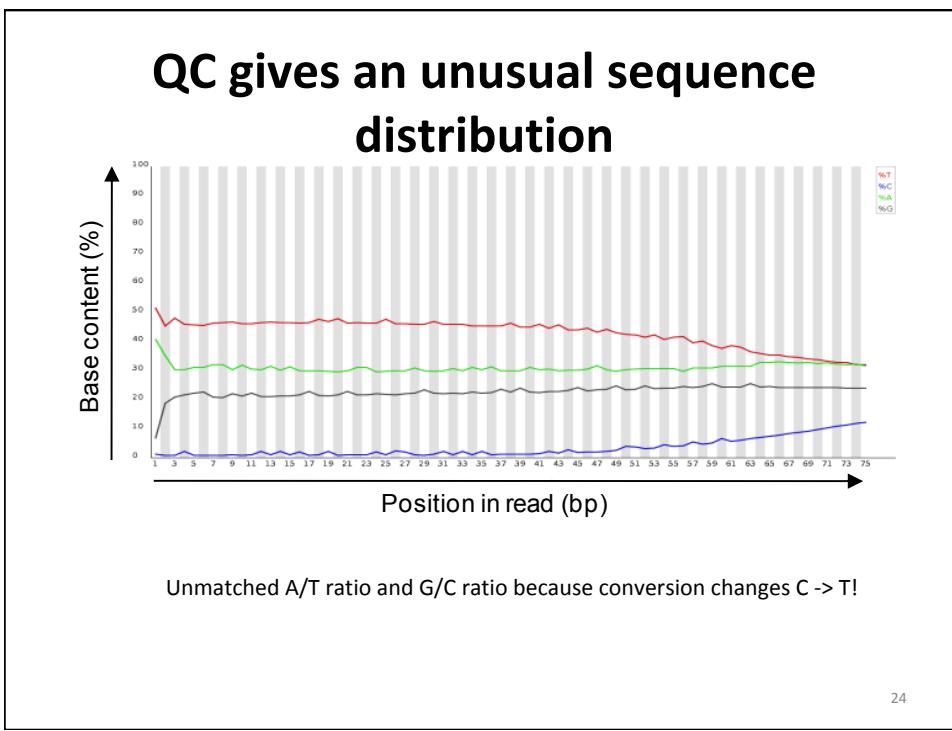
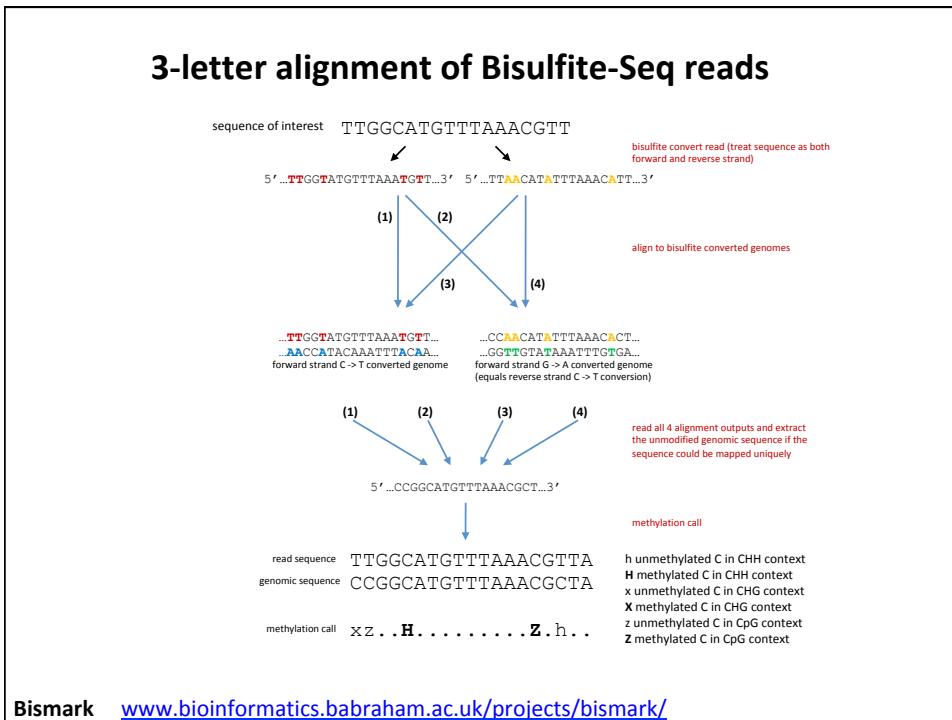
### non-CG context



60%-80% of all CpGs are methylated



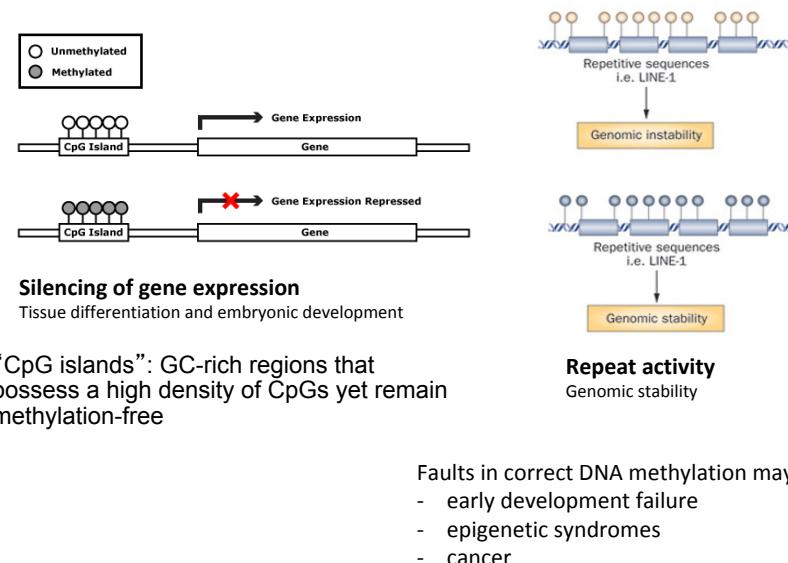




## DNA methylation is not distributed evenly in the mammalian genome

- In human somatic cells, 60%-80% of all CpGs (~1% of total DNA bases) are methylated
  - Most methylation is found in “repetitive” elements
- “CpG islands”, GC-rich regions that possess a high density of CpGs, remain methylation-free
  - The promoter regions of ~70% of genes are embedded in CpG islands

## Regulation by DNA methylation



## Mechanisms of gene silencing by methylation

### Direct mechanism:

Inhibition of transcription factor binding (eg.CTCF, UBF)

Not a universal mechanism since not all transcription factor binding sites contain CG dinucleotides

### Indirect mechanism:

Inhibition mediated by methyl-CpG binding proteins MeCP1/ MeCP2

Recruitment of corepressor complexes including histone deacetylases (HDAC)

Change in chromatin conformation

## Differences in DNA methylation patterns between identical twins increase with aging

