

BIO306: Bioinformatics

Lecture 12

Epienetics and epigenome

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Review last lecture

- What's the advantage of RNA-seq compared with microarray?
- What factors should we consider for RNA-seq data normalization?
- What's the advantage of single cell sequencing over bulk cells?

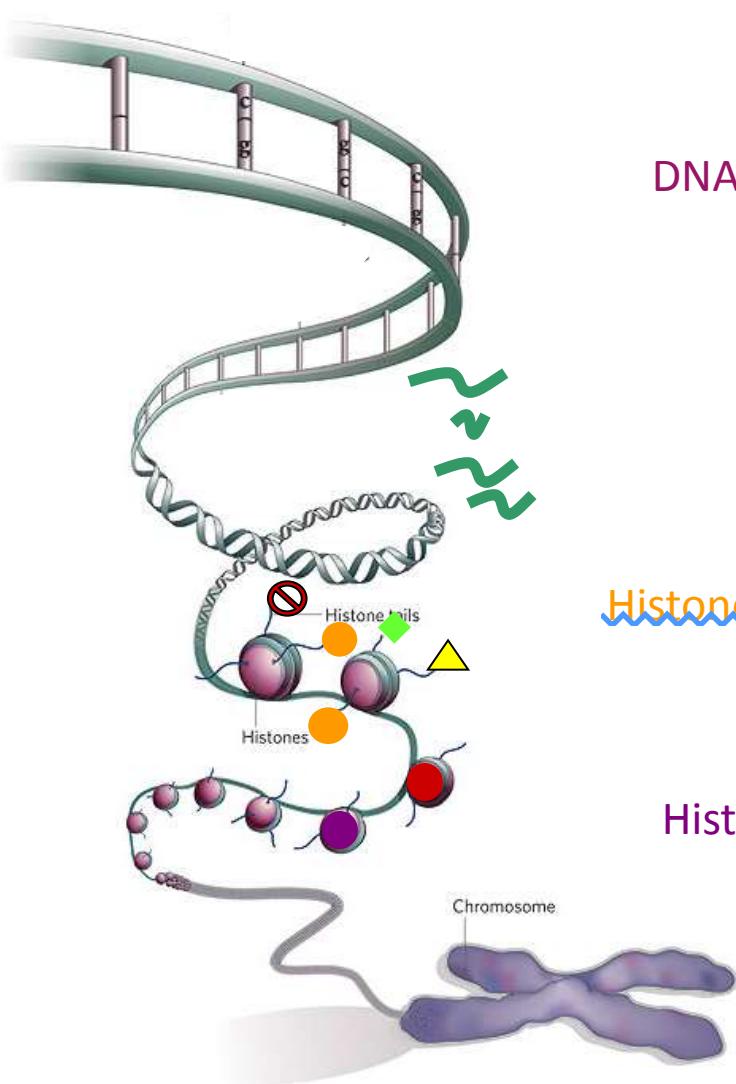
Origin of the term ‘epigenetics’

- C. H. Waddington coined the term epigenetics in 1942 as pertaining to epigenesis. Epigenesis in the context of the biology of that period referred to the differentiation of cells from their initial totipotent state in embryonic development.
- When Waddington coined the term, the physical nature of genes and their role in heredity was not known; he used it as a conceptual model of how genes might interact with their surroundings to produce a phenotype; he used the phrase "epigenetic landscape" as a metaphor for biological development.

Definition of epigenetics

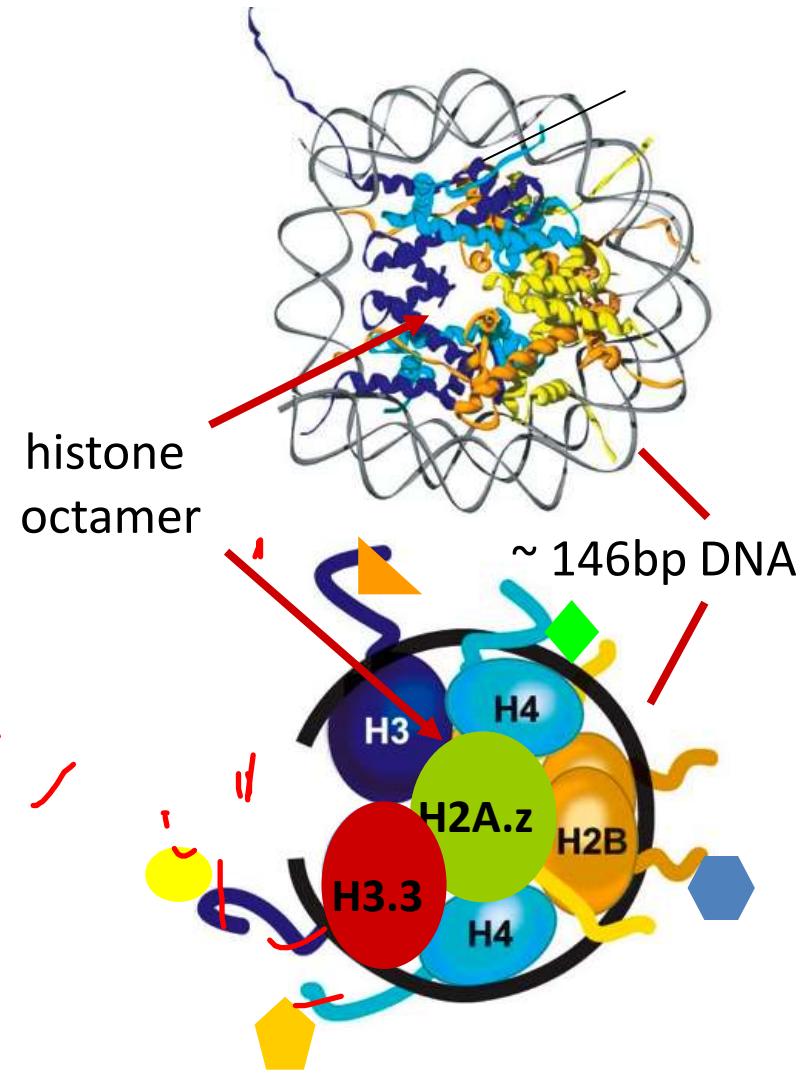
- Definition: Epigenetics is the study of heritable changes in gene function that do not involve changes in the DNA sequence.
- Epigenetics most often denotes changes in a chromosome that affect gene activity and expression.
- The standard definition of epigenetics requires these alterations to be heritable, either in the progeny of cells or of organisms.

How epigenomes mark the genome?

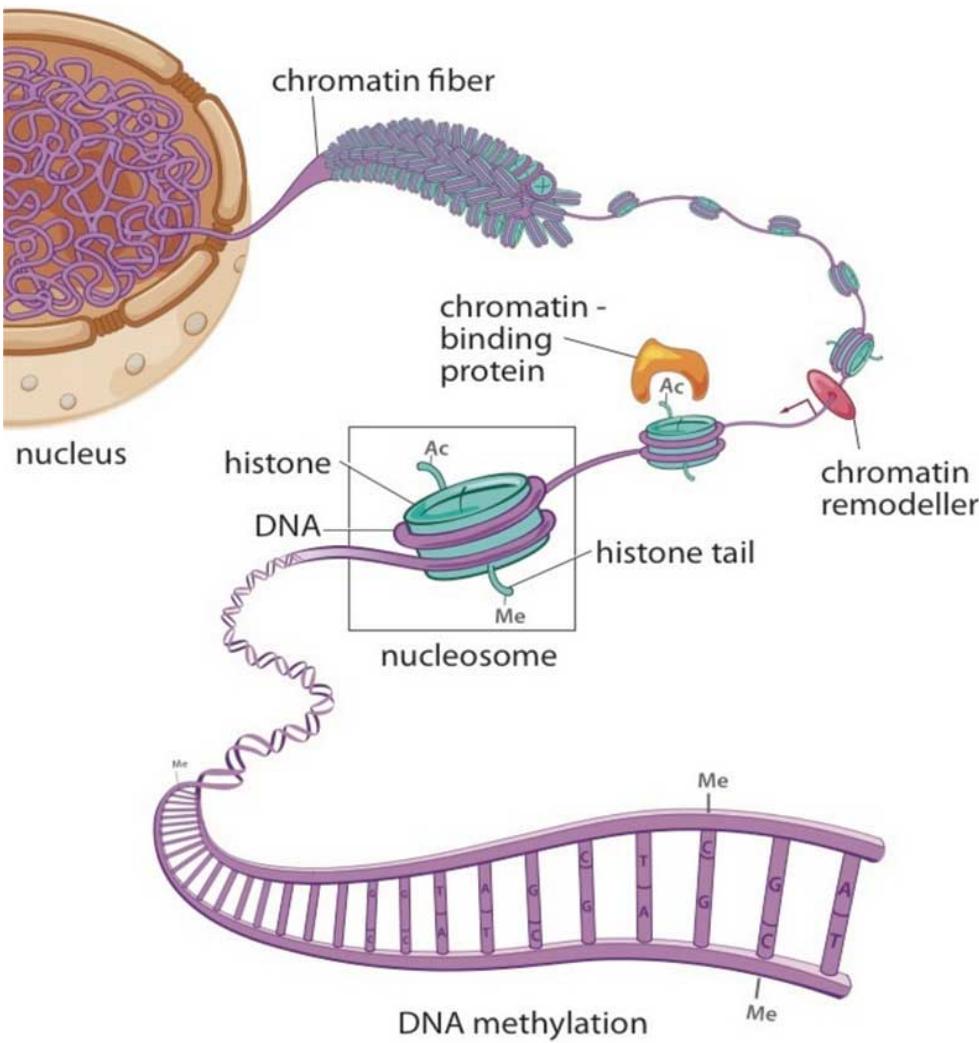


DNA methylation
smRNAs
Histone modifications

Histone variants



Epigenetics categories



DNA modification
DNA cytosine methylation

Chromatin related change
Histone modifications
Histone variants
Nucleosome positioning
Chromatin accessibility
Structure of chromatin
Higher order structure

RNA
mRNA modification
Non-coding RNA (smRNAs)

Protein
Prion
Transcription factor binding

Epigenetics assays using NGS

- Bisulfite-Sequencing (BS-seq)
 - DNA methylation
- MNase-seq
 - Nucleosome positioning
- Chromatin Immunoprecipitation sequencing (ChIP-seq)
 - Signatures of protein association
 - Histone variants and histone modification
- DNase-seq, ATAC-seq
 - Chromatin accessibility
- Hi-C, CHIA-PET
 - Chromatin architecture

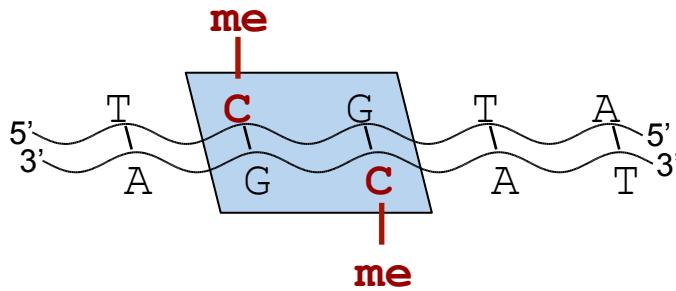
Types of DNA methylation

canonical
**non-canonical
(mammals)**

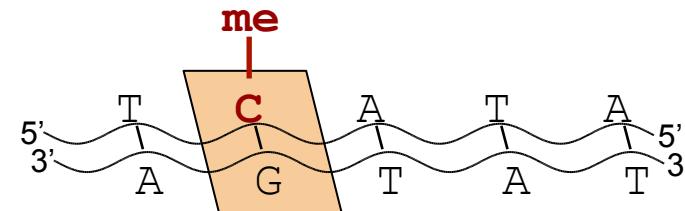
	Plants	Mammals
CG	symmetric	symmetric
CHG	symmetric	asymmetric
CHH	asymmetric	asymmetric

$$H = T/A/C$$

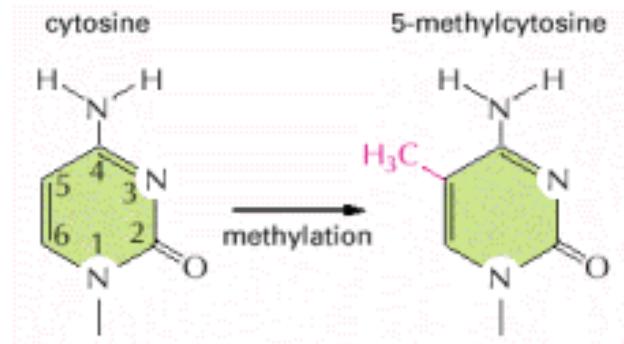
CG context



non-CG context

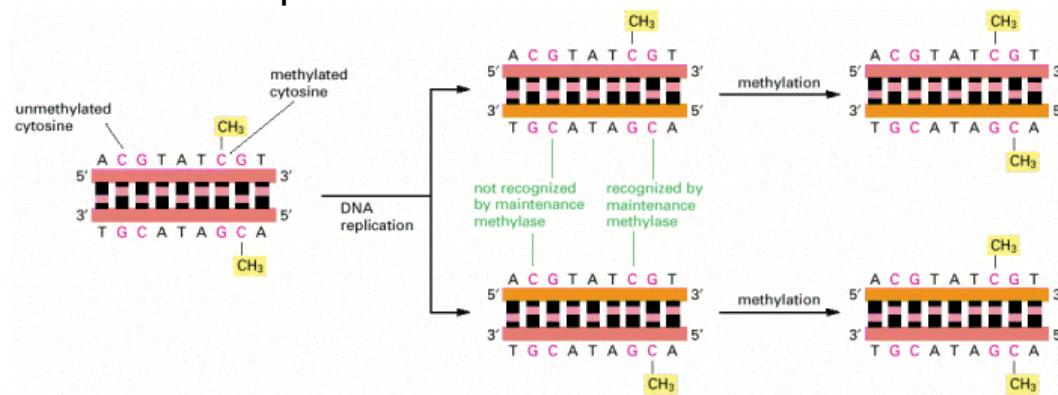


Canonical DNA methylation is inheritable



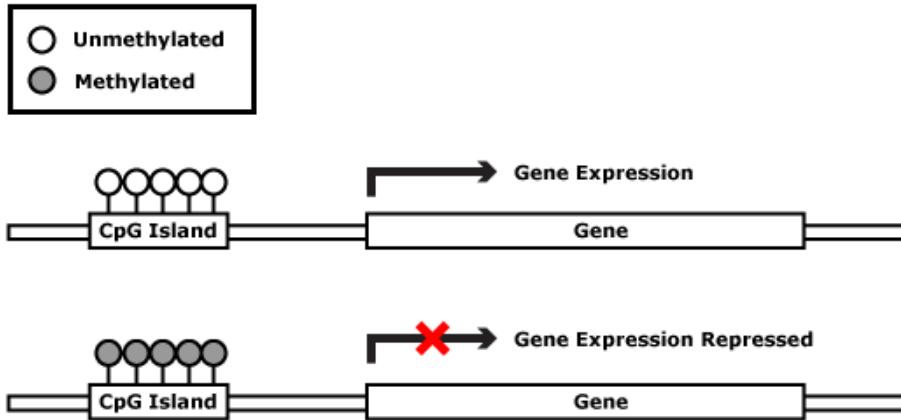
Mammals:

- Cytosine methylation
- Gene regulation
- CpG islands

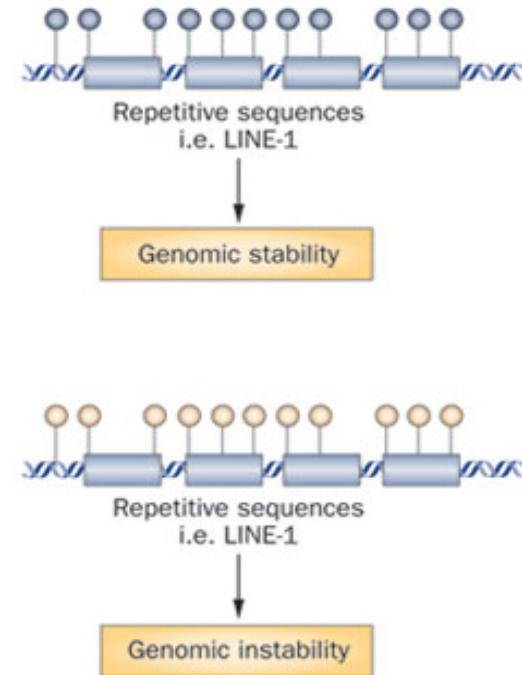


Cytosine methylation is passed on to daughter cells

Regulation by DNA methylation



Silencing of gene expression
Tissue differentiation and embryonic development

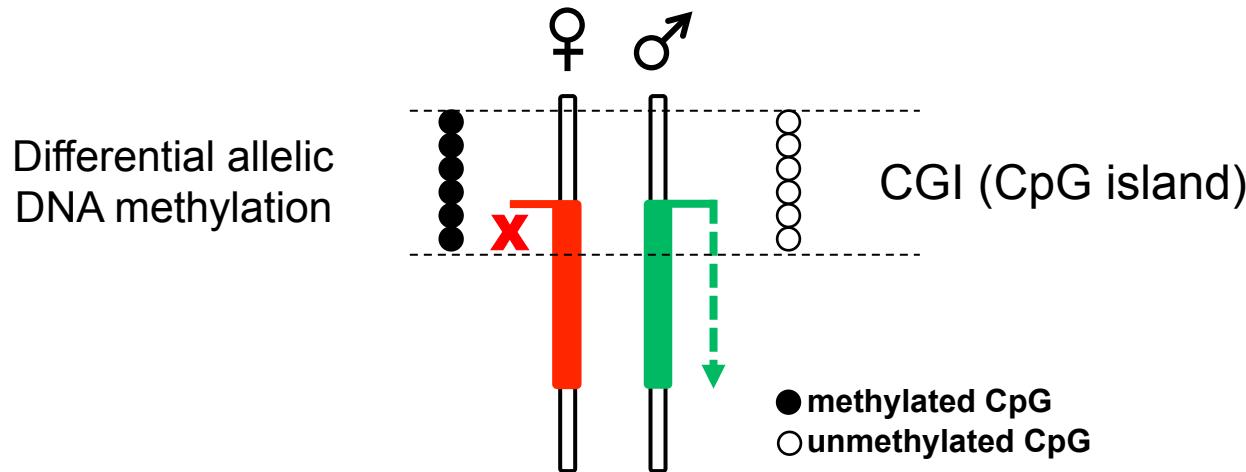


Repeat activity
Genomic stability

Faults in correct DNA methylation may result in

- early development failure
- epigenetic syndromes
- cancer

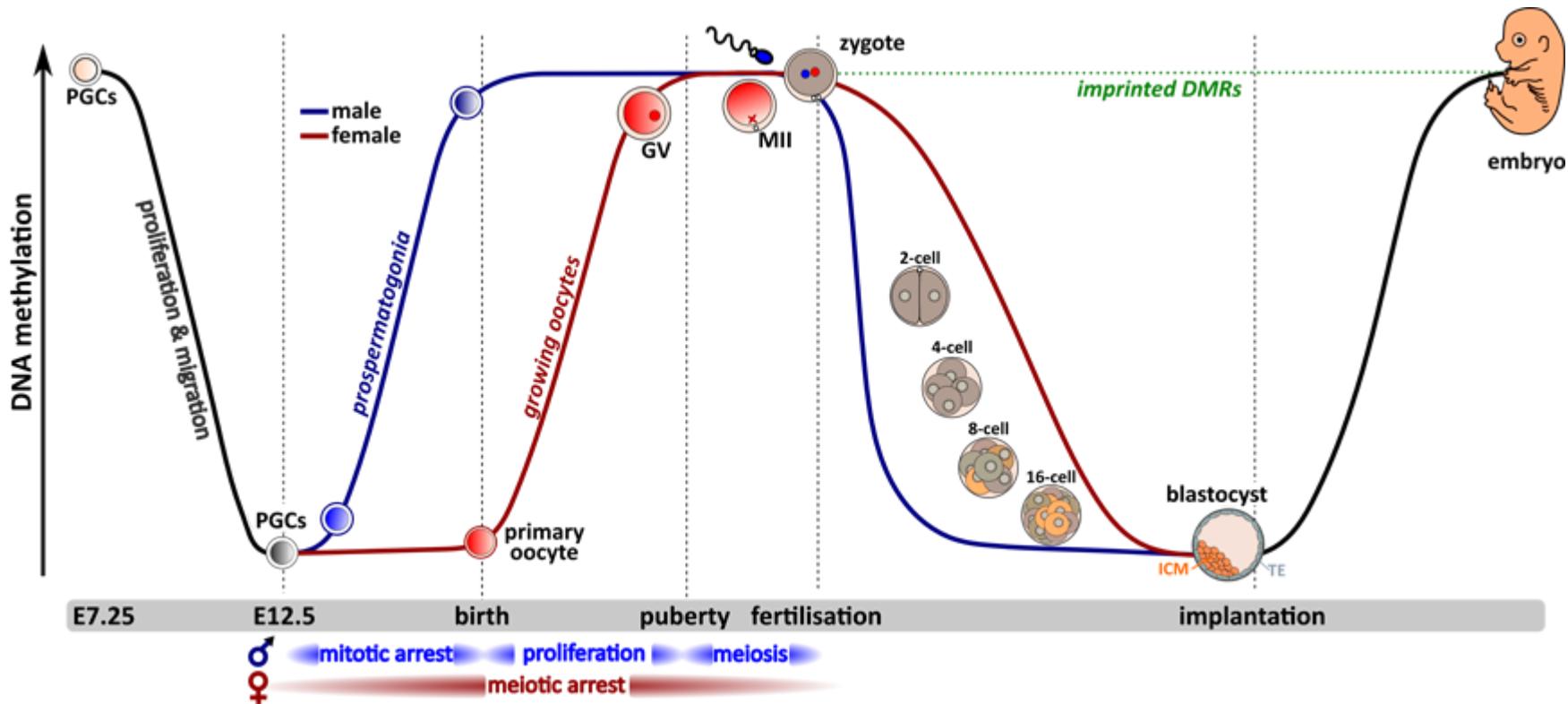
Imprinted Genes: mono-allelic expression



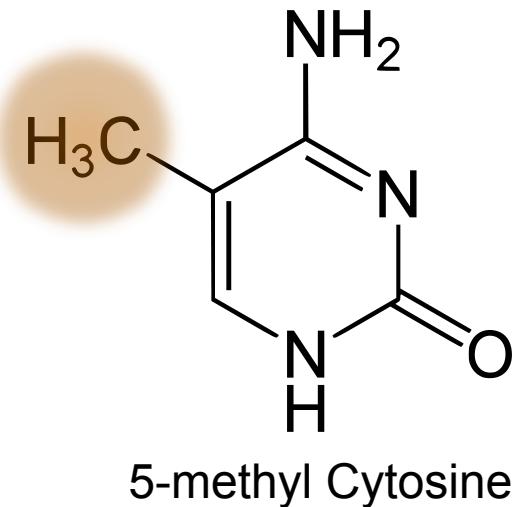
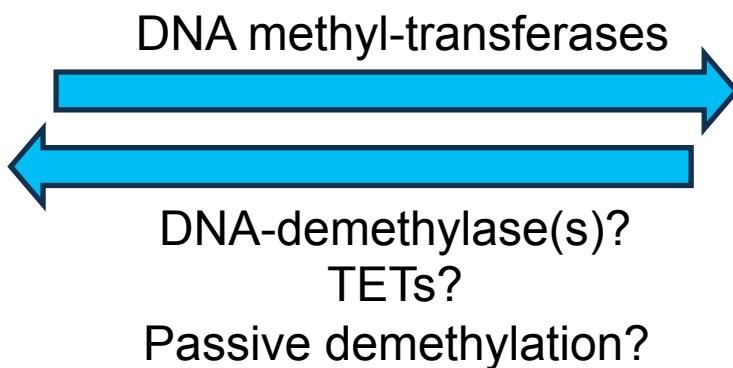
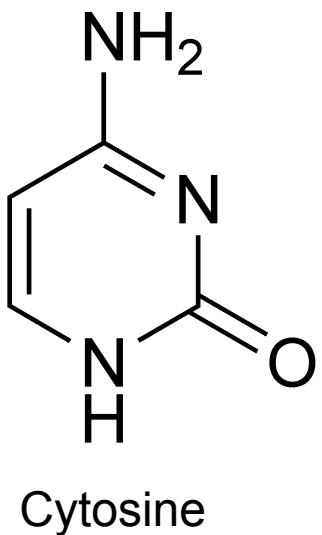
Imprinted Genes: Mono-allelic expression with parent-of-origin specificity.

Have key roles in energy metabolism, placenta functions.

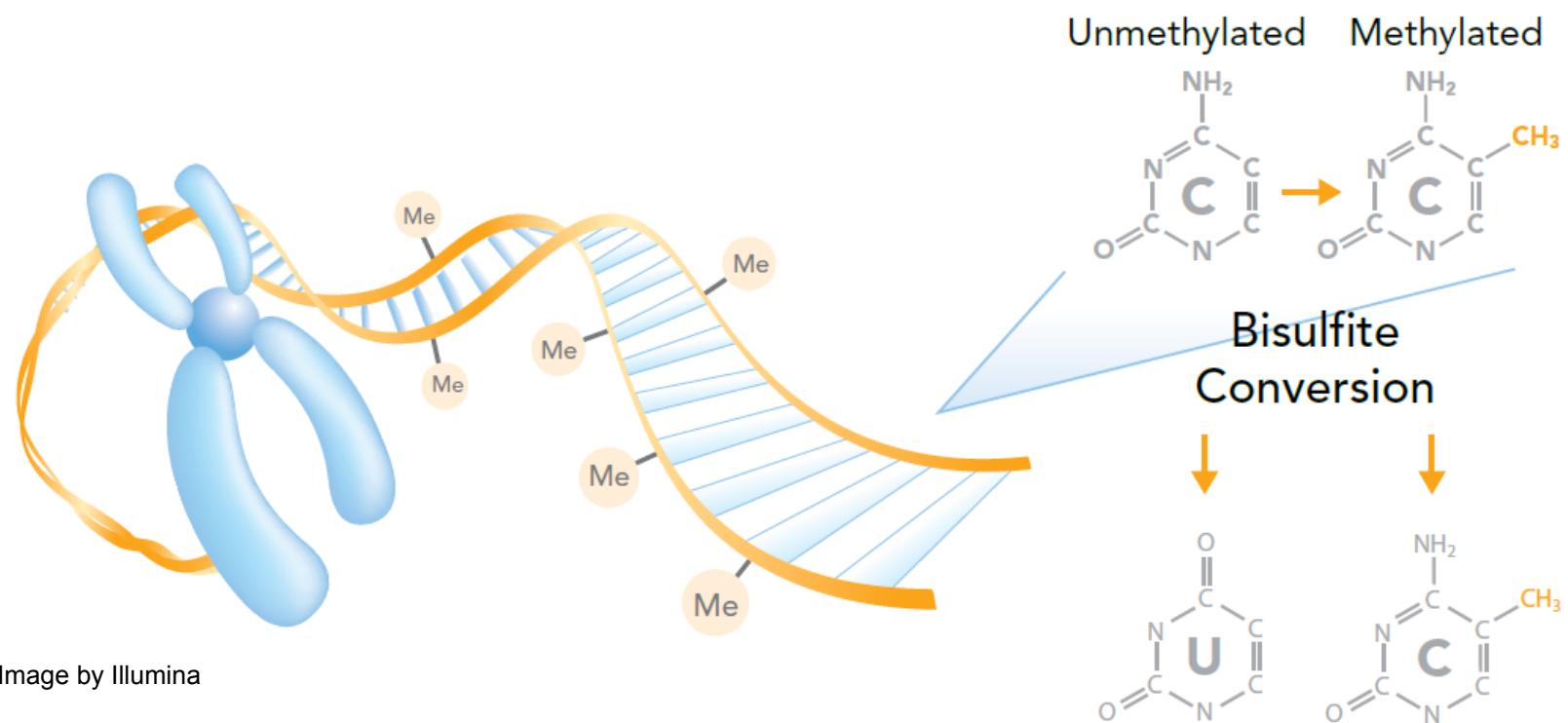
DNA methylation is reset during reprogramming



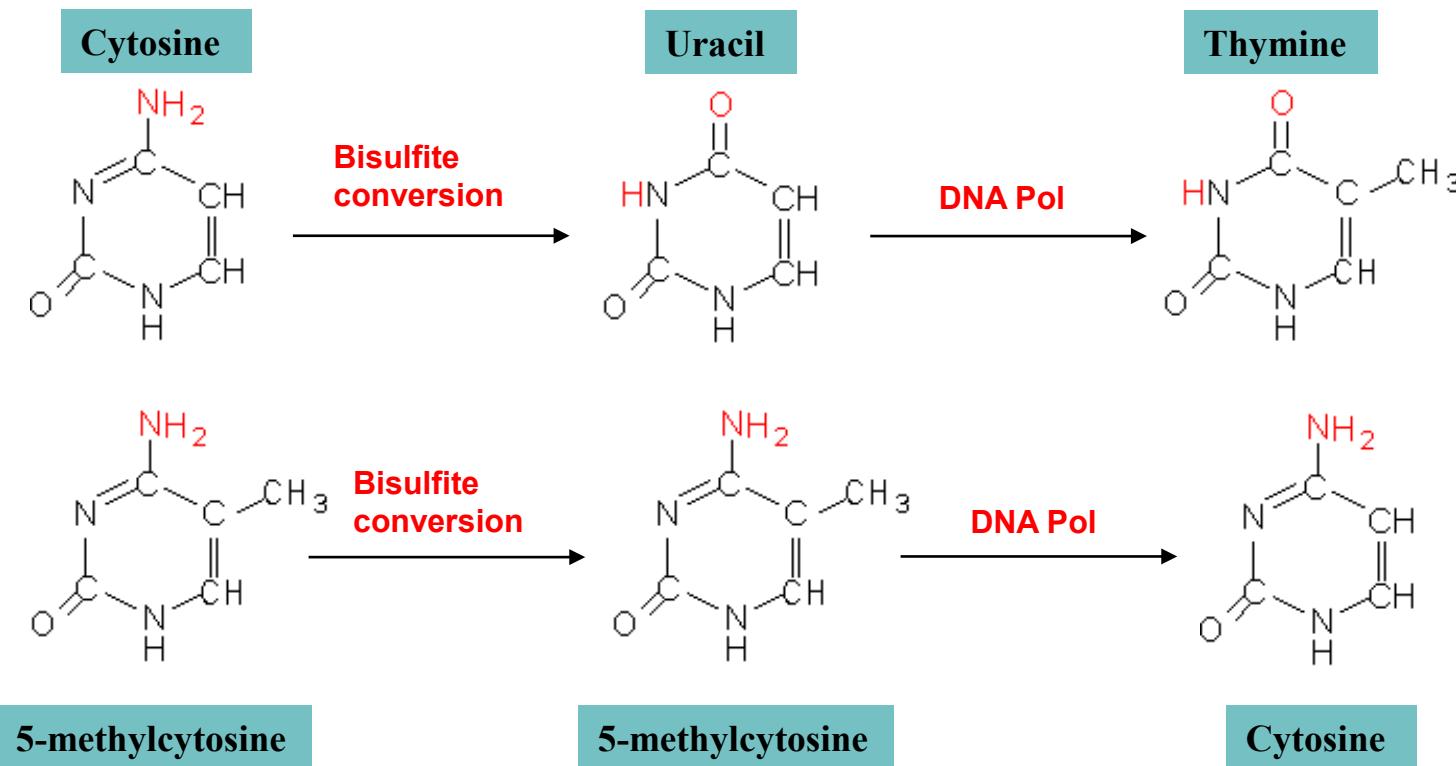
DNA methylation and demethylation



Measuring DNA methylation by Bisulfite-sequencing



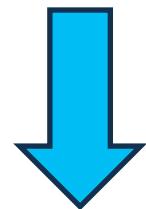
DNA Methylation with NGS : Bisulfite Conversion



>>A C^m G T T C T C C A G T C>>
↓
Bisulfite conversion
>>A C^m G T T T T T A G T T>>

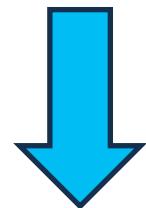
Bisulfite Informatics

CCAGTCGCTATAGCGCGATATCGTA
me me



Convert

TTAGT TGC TATAG TGCGATATTGTA

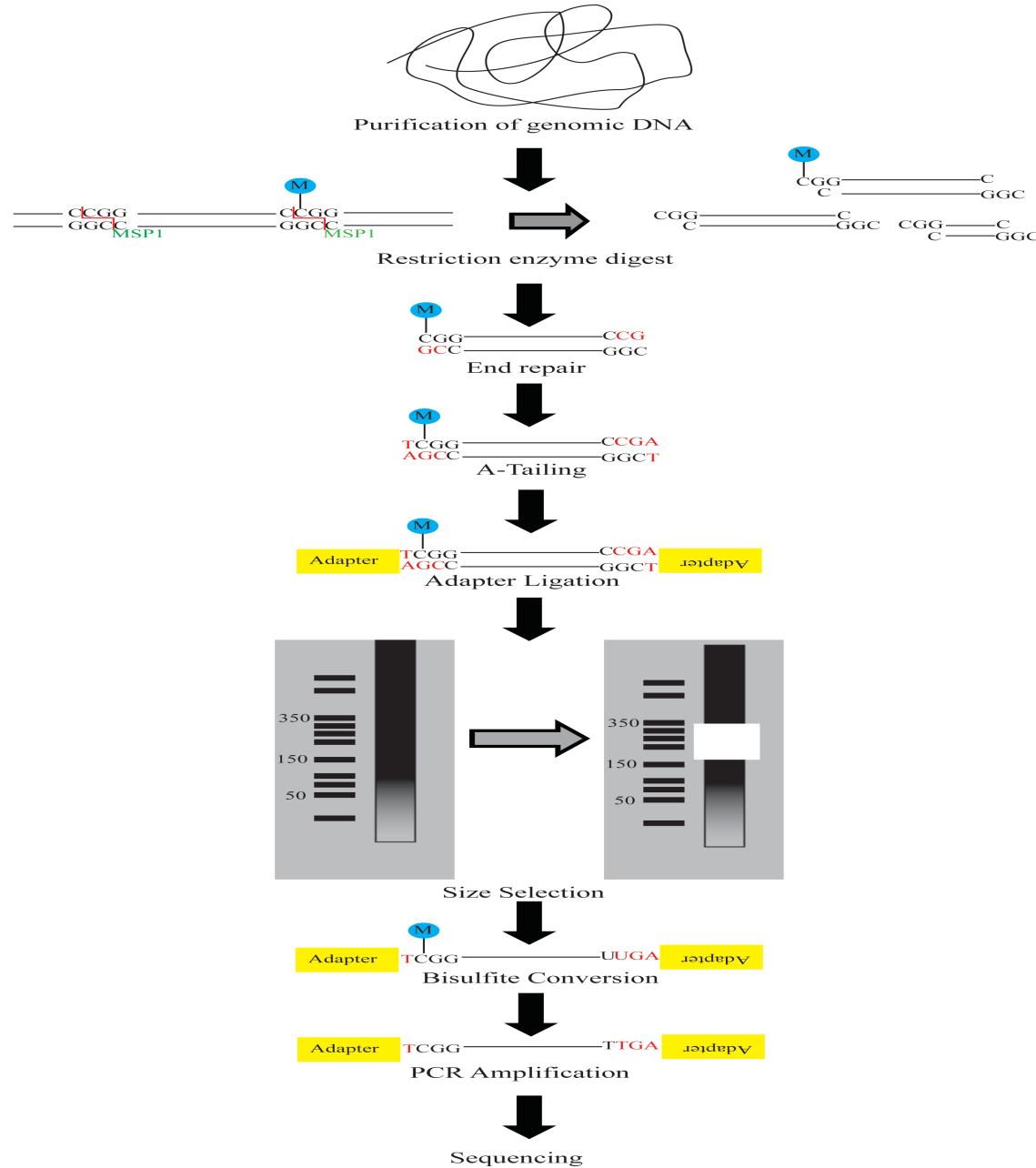


Map

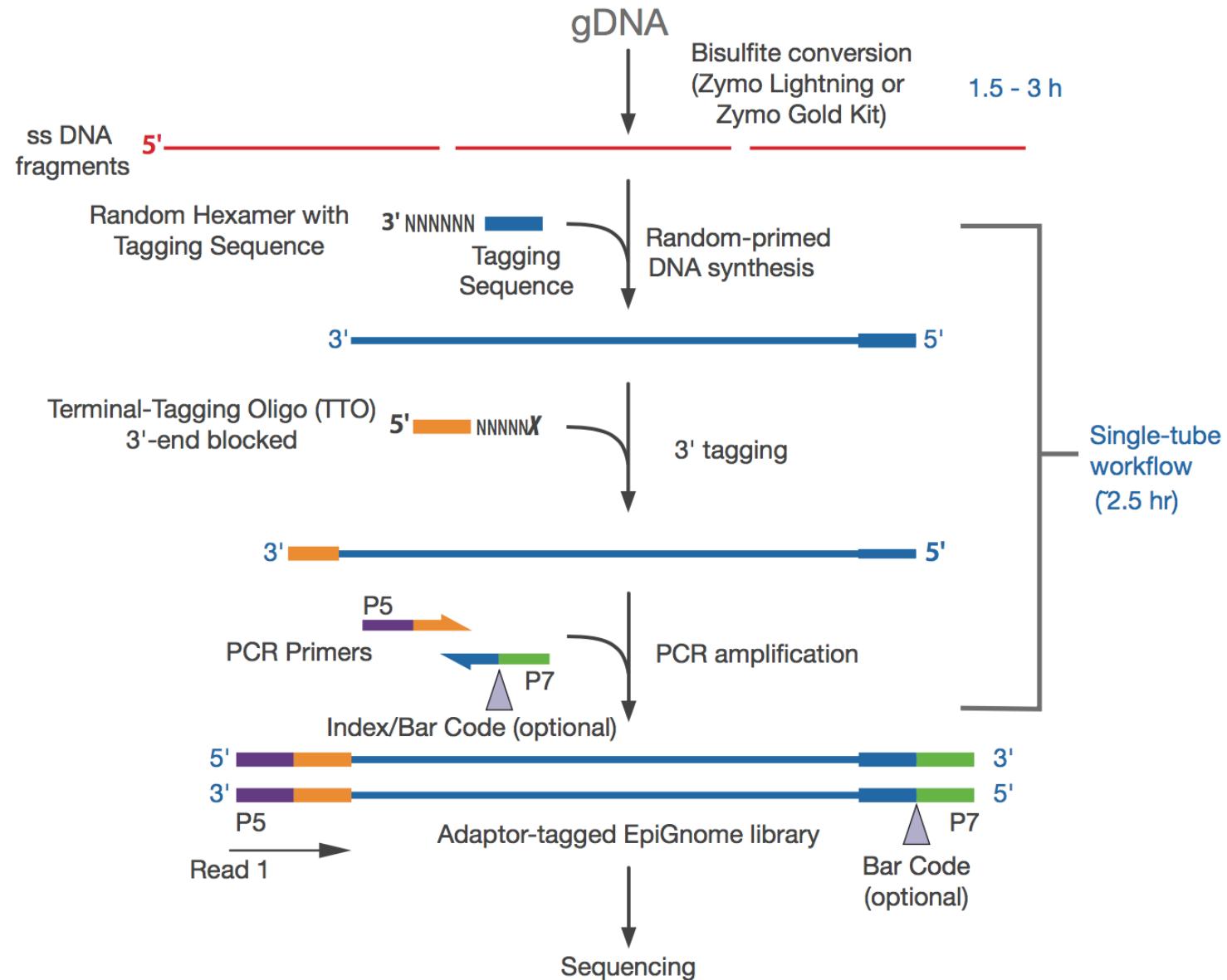
TTAGTTGCTATAGTGCGATATTGTA

||| ||| | | | | | | | | | | | |
... CCAGTCGCTATAGCGCGATATCGTA ...

Reduced representation BS-Seq (RRBS)

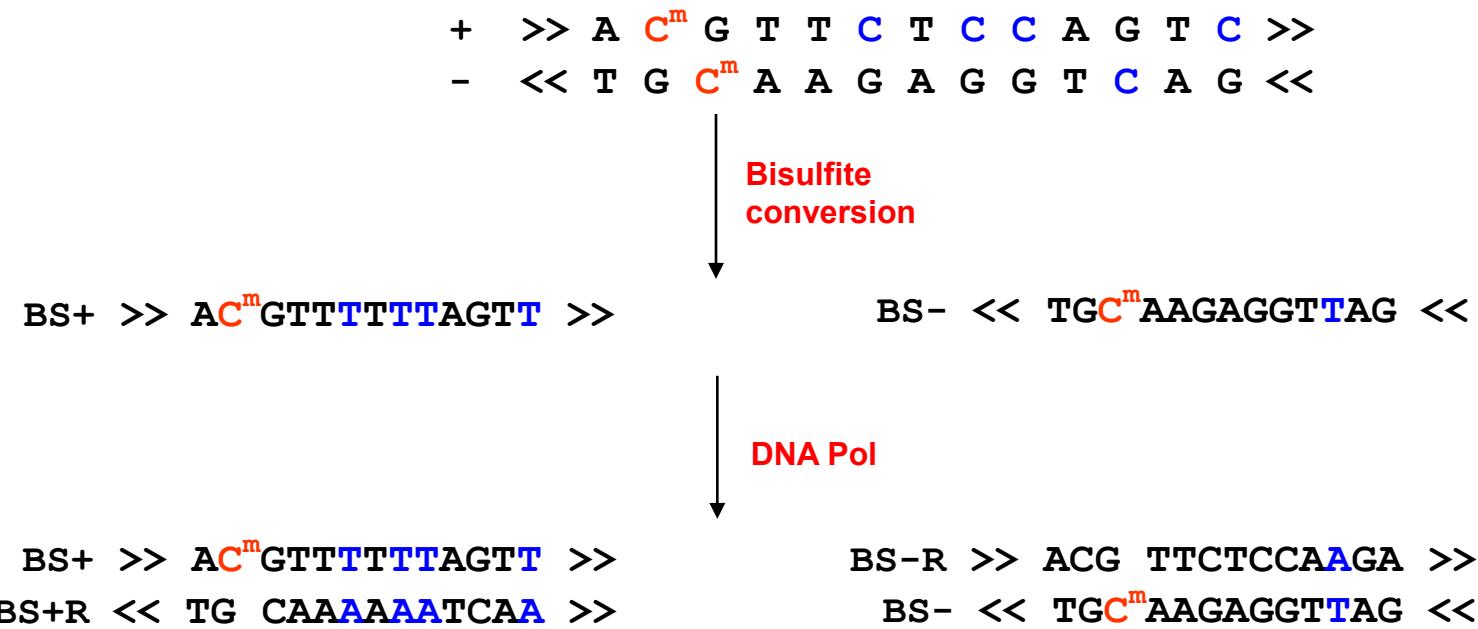


Whole Genome Bisulfite Sequencing (WGBS)



Mapping Bisulfite-converted reads

- Mutated sequence, increased search space



- Specialized aligners
 - BISMA, BSMAP, BS Seeker, MAQ bisulfite mode

Bismark workflow

Pre Alignment

FastQC Initial quality control
Trim Galore Adapter/quality trimming using Cutadapt; handles RRBS and paired-end reads; Trim Galore and RRBS User guide

Alignment

Bismark Output BAM

Post Alignment

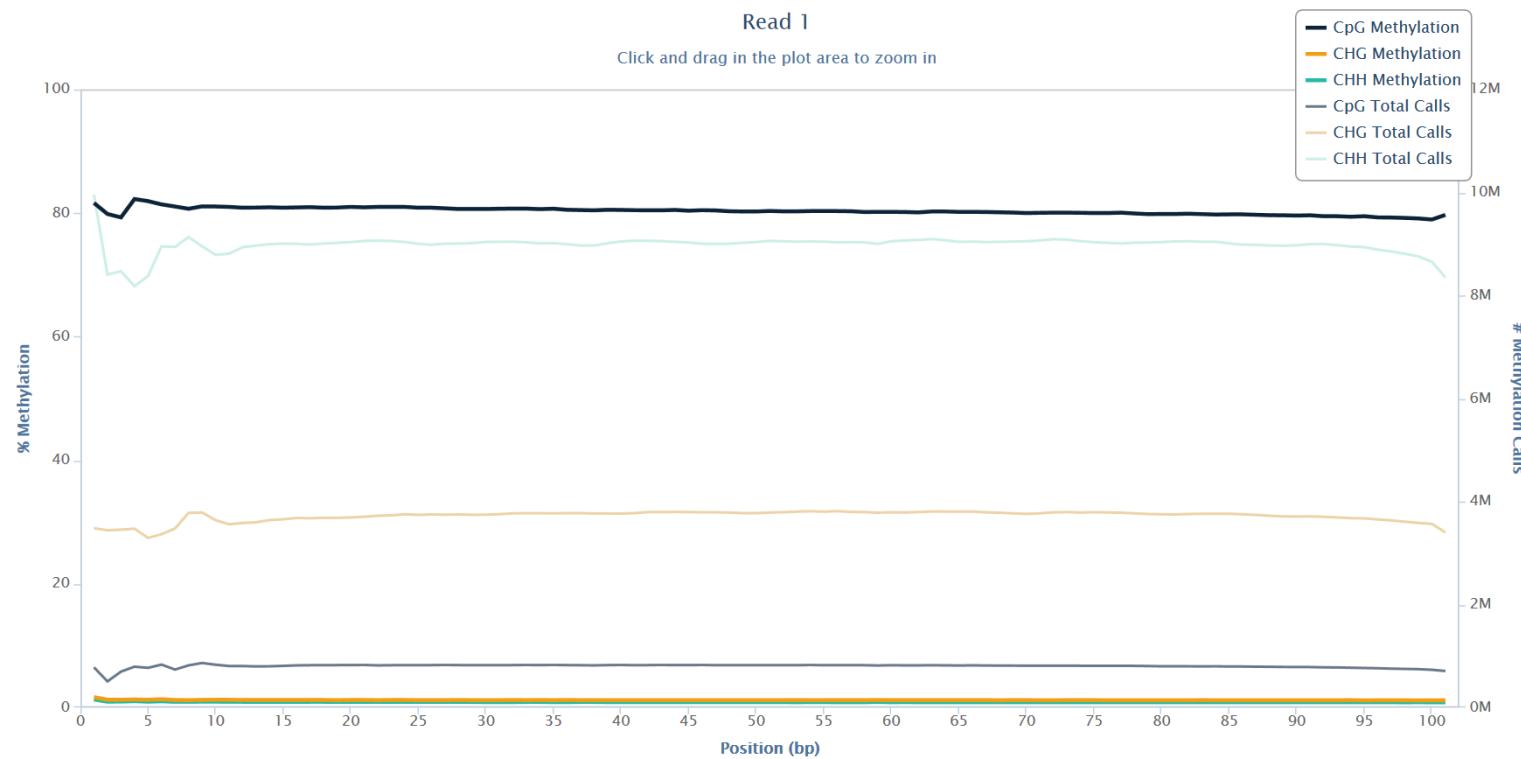
Deduplication optional
Methylation extractor Output individual cytosine methylation calls; optionally bedGraph or genome-wide cytosine report
 M-bias analysis
bismark2report Graphical HTML report generation
Example: http://www.bioinformatics.babraham.ac.uk/projects/bismark/PE_report.html



protocol: *Quality Control, trimming and alignment of Bisulfite-Seq data*

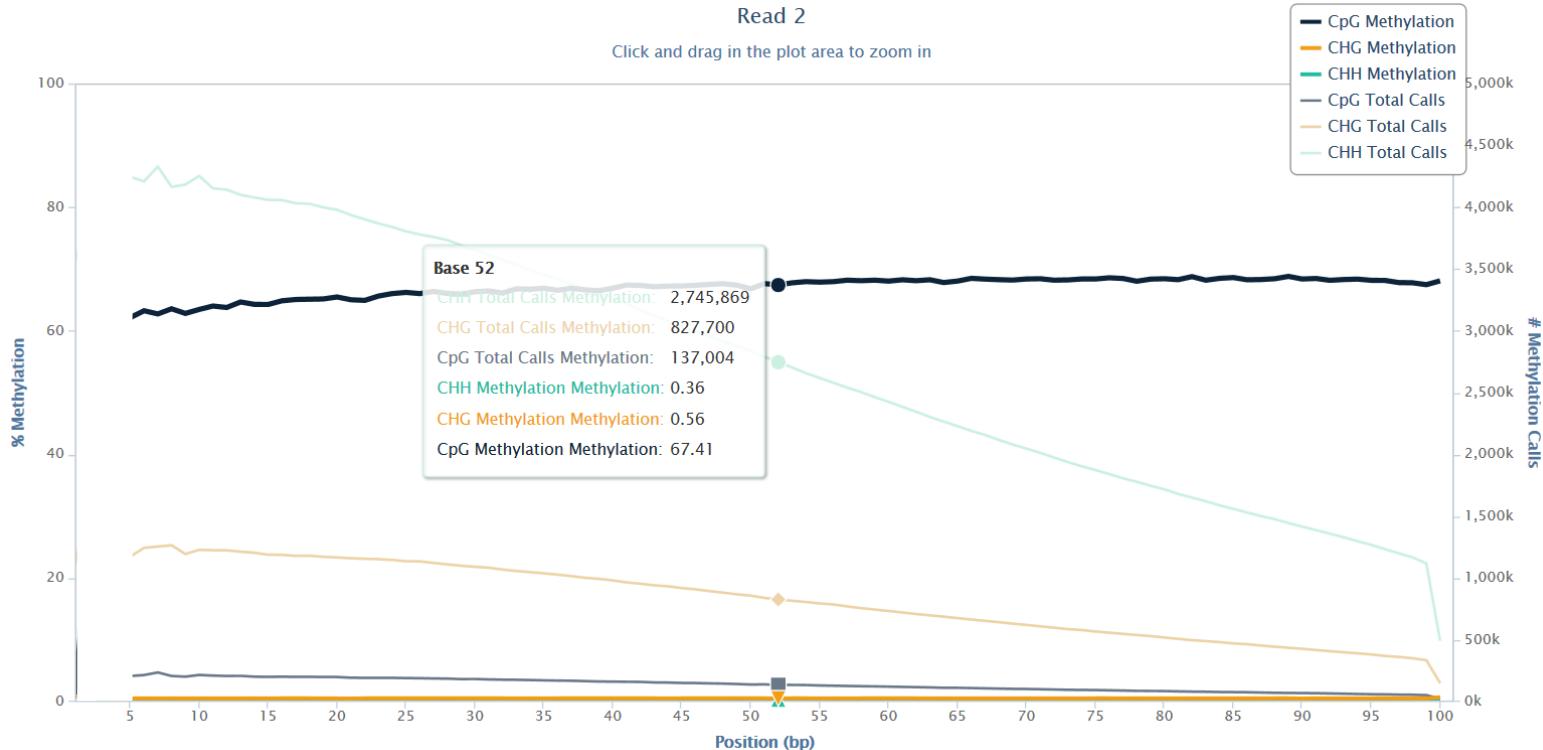
M-Bias Plot

Mapped QC - Methylation bias



good opportunity to look at conversion efficiency

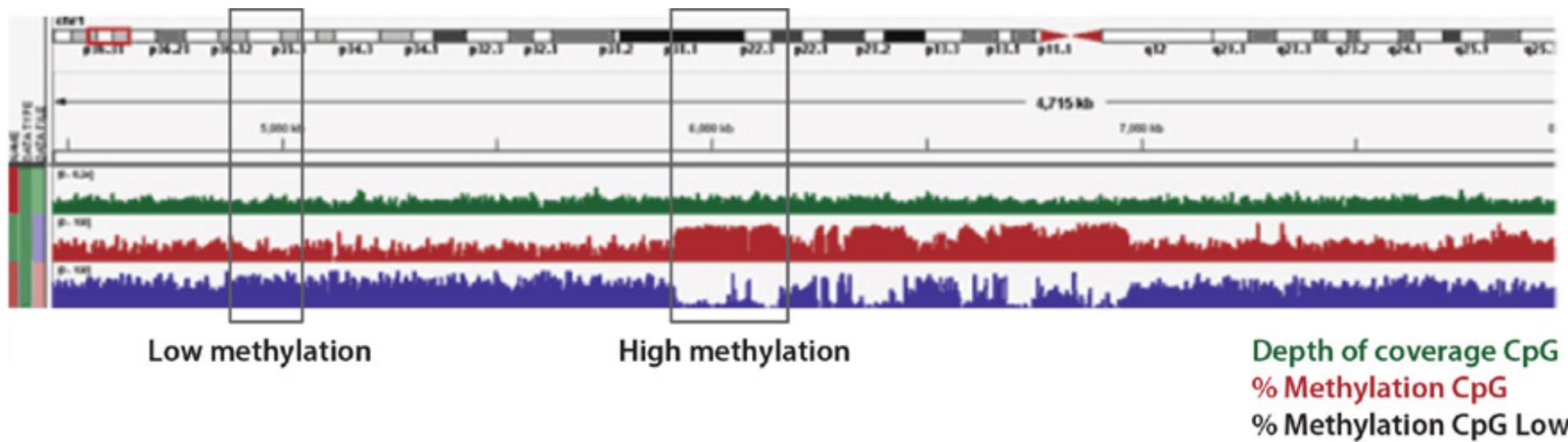
Artificial methylation calls in paired-end libraries



end repair + A-tailing



CpG Methylation Coverage

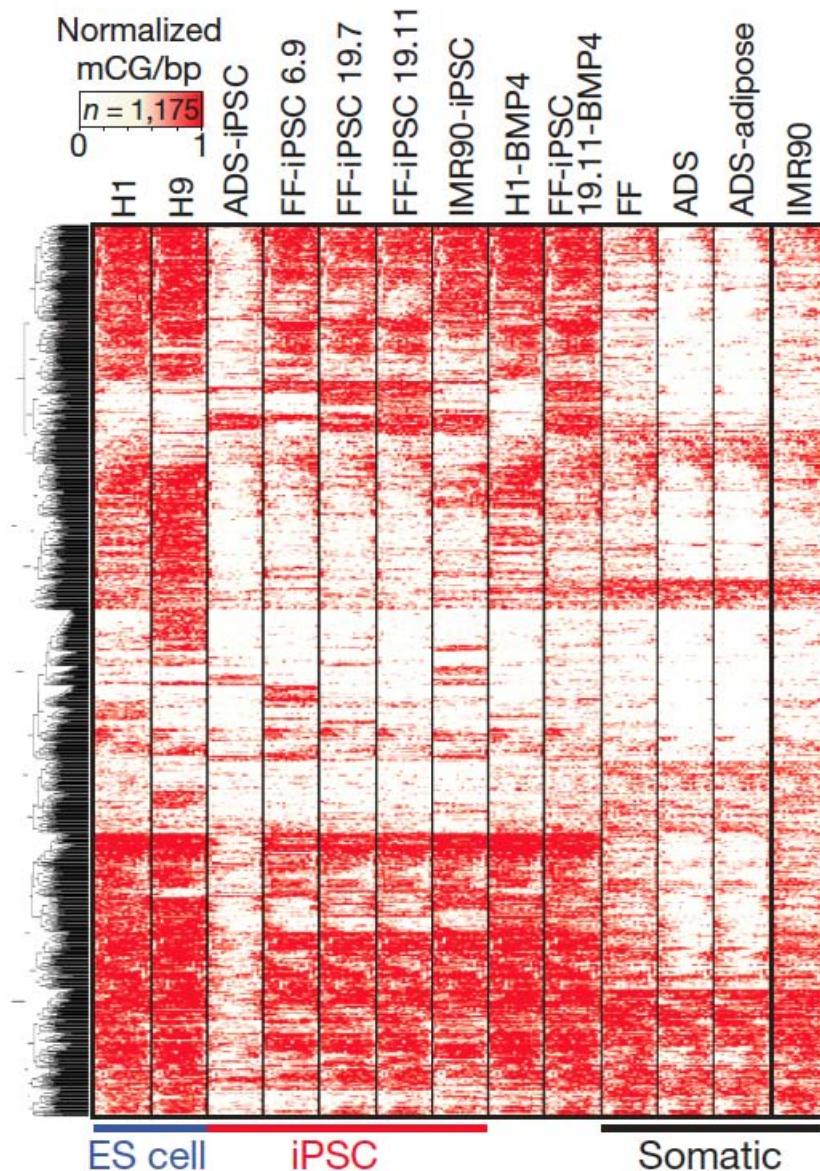
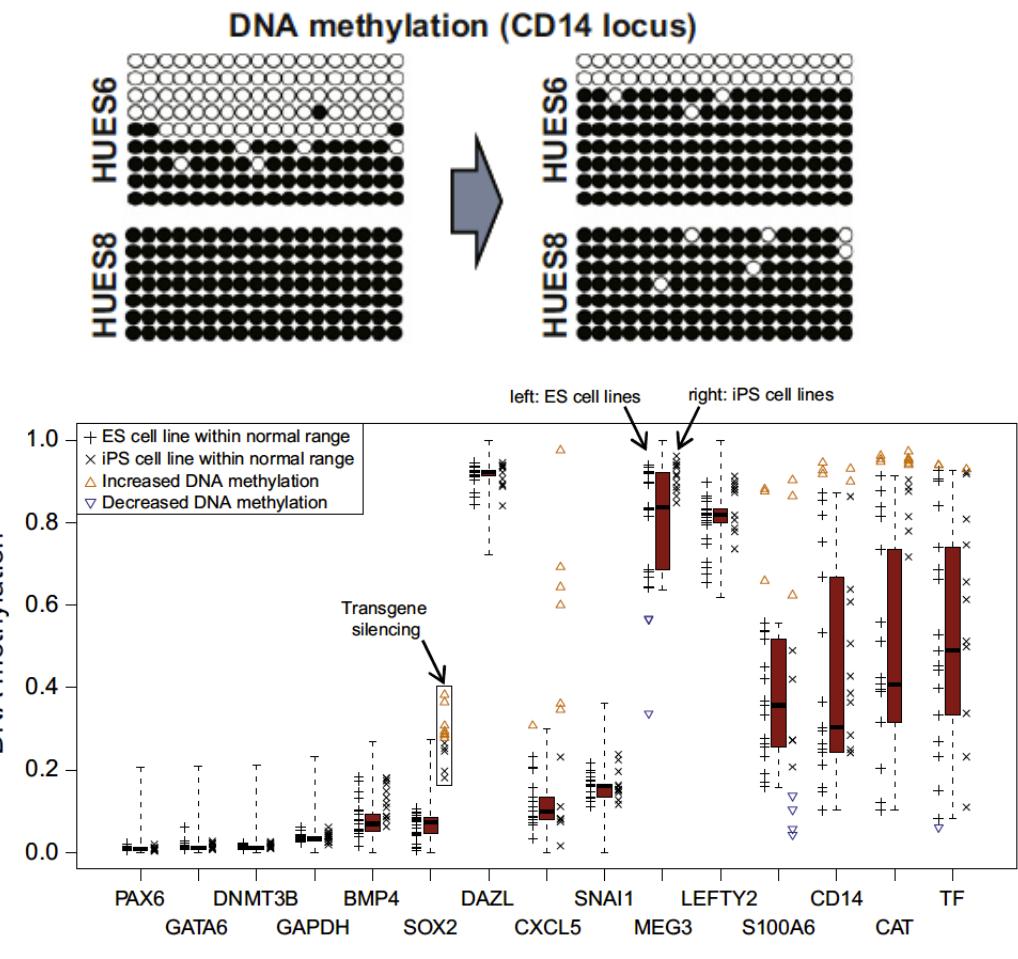


DNA Methylation : ES – iPS comparison

Bock *et al.*, Cell 2011

Lister *et al.*, Nature 2010

- CpG status across individual allele copies, variability

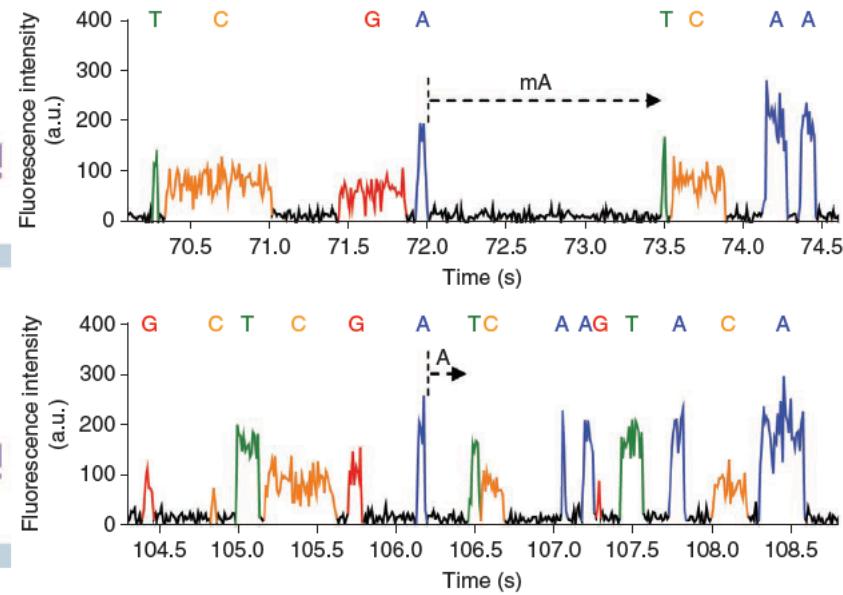
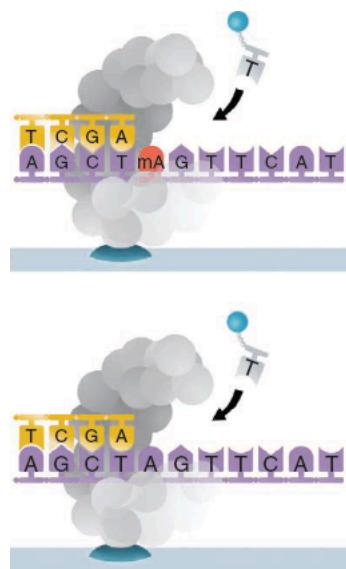
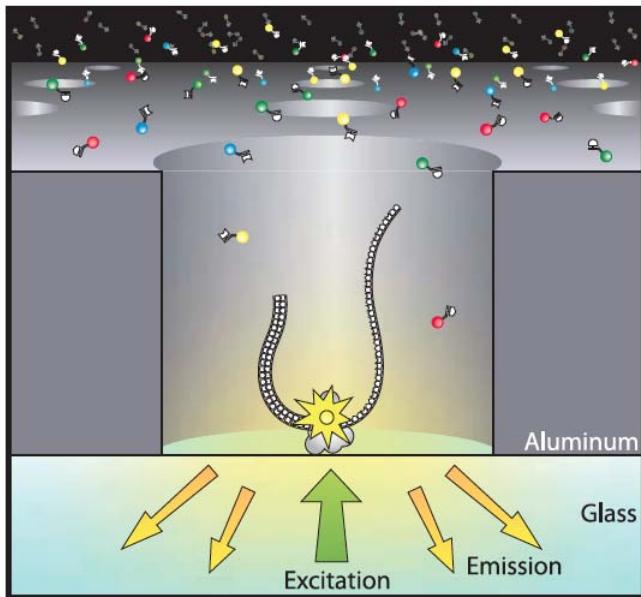


DNA Methylation : direct assessment

Direct detection of DNA methylation during single-molecule, real-time sequencing

Nature Methods 2010

Benjamin A Flusberg, Dale R Webster, Jessica H Lee, Kevin J Travers, Eric C Olivares, Tyson A Clark, Jonas Korlach & Stephen W Turner

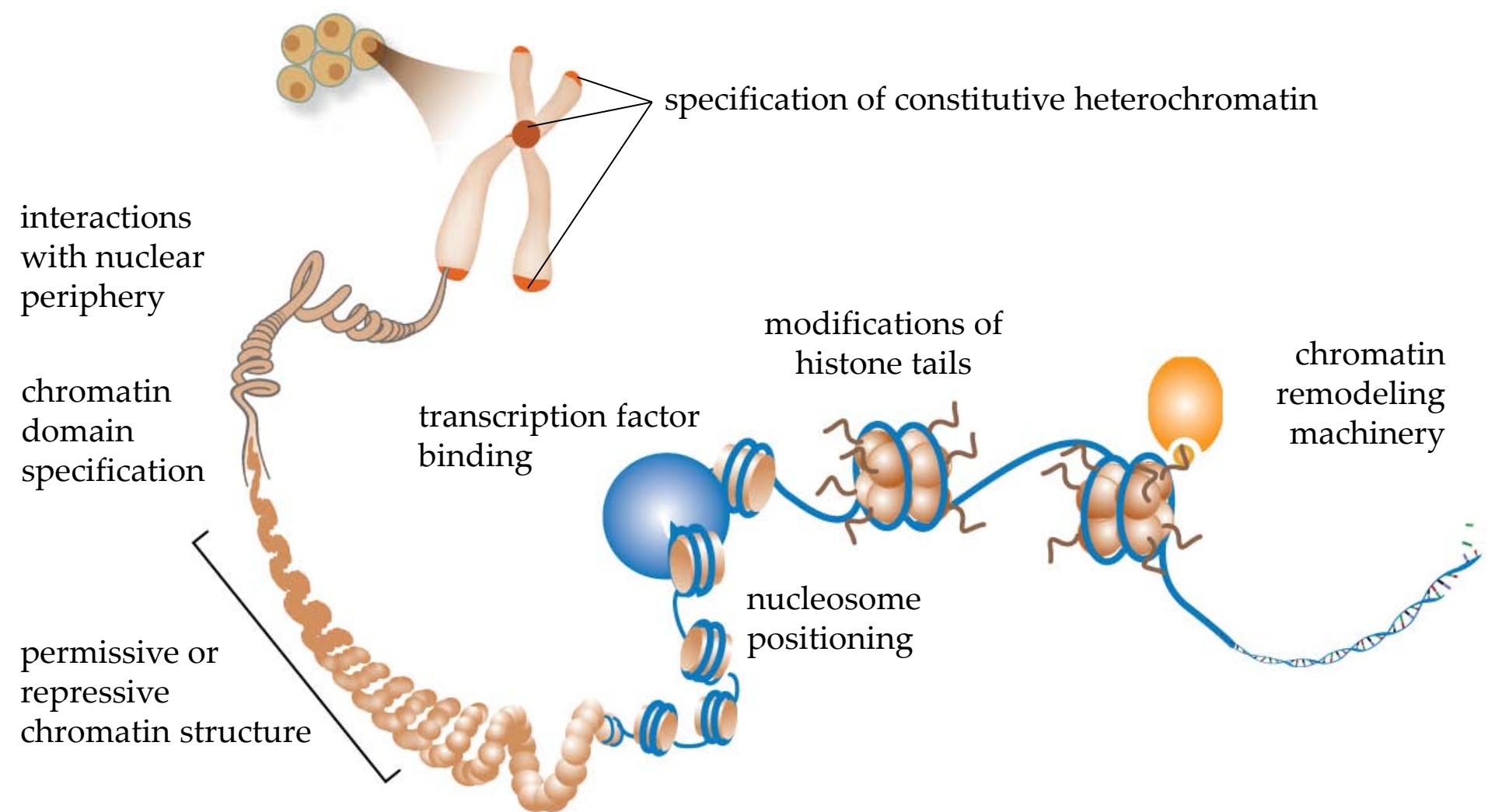


- PacBio Real-time sequencing technology, ZWG, circular templates
- Interpulse duration differences, complex dependency
- Unmethylated references, *de novo*

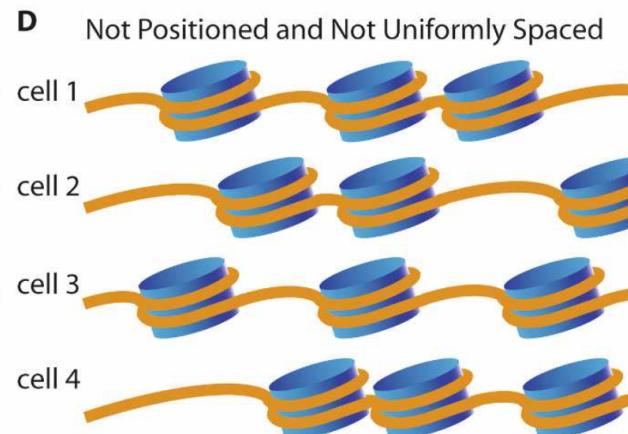
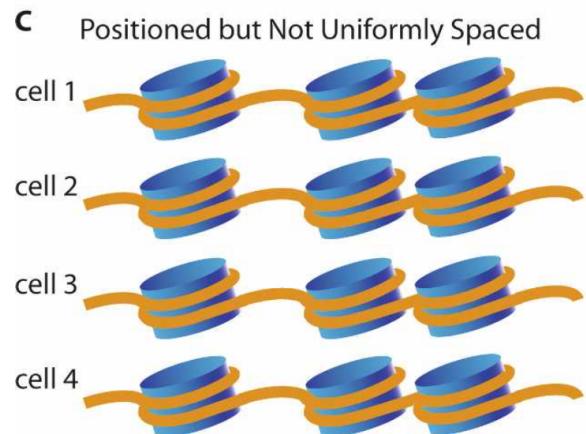
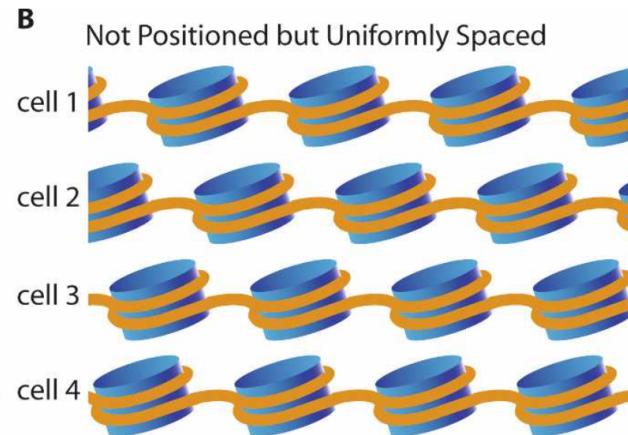
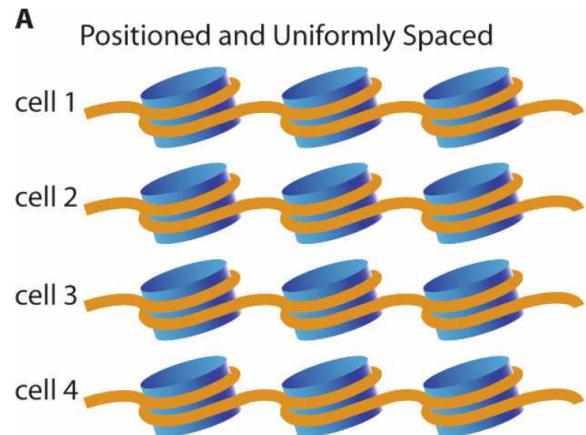
Epigenetics assays using NGS

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Chromatin structure and regulation

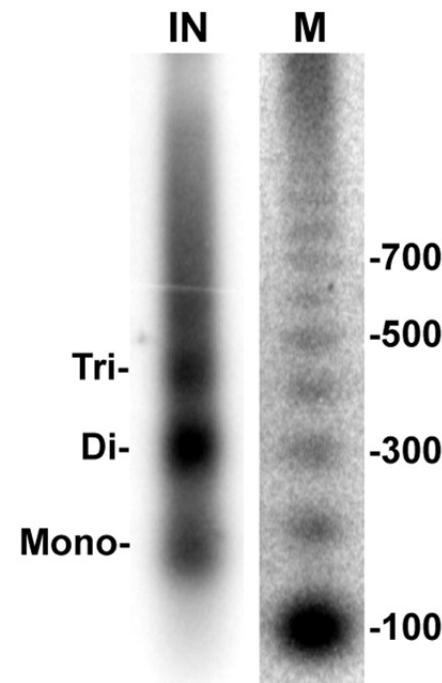
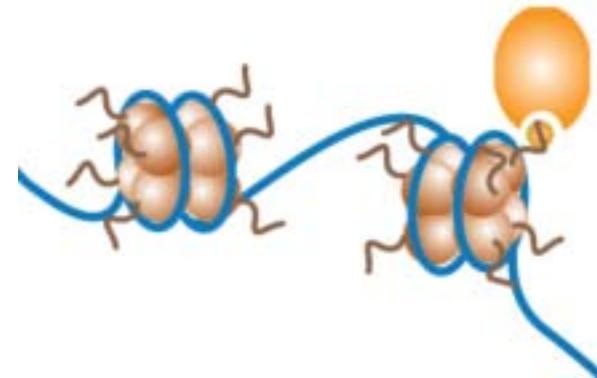


Possible models of nucleosome positioning



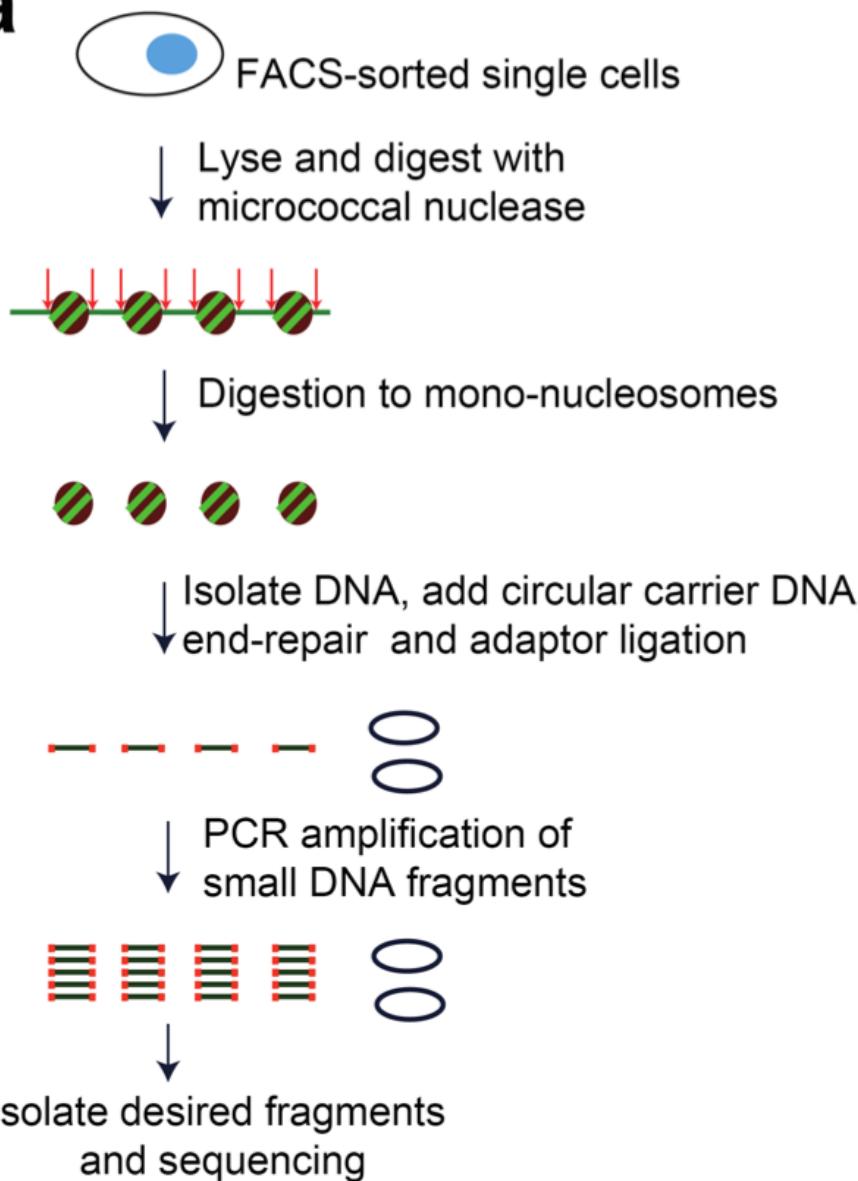
Nucleosome Positioning

- Which DNA fragments wrapped around nucleosomes?
- Digest linker DNA with Micrococcal Nuclease (MNase)
- Isolate mono-nucleosomal fragments
- Construct library and sequence



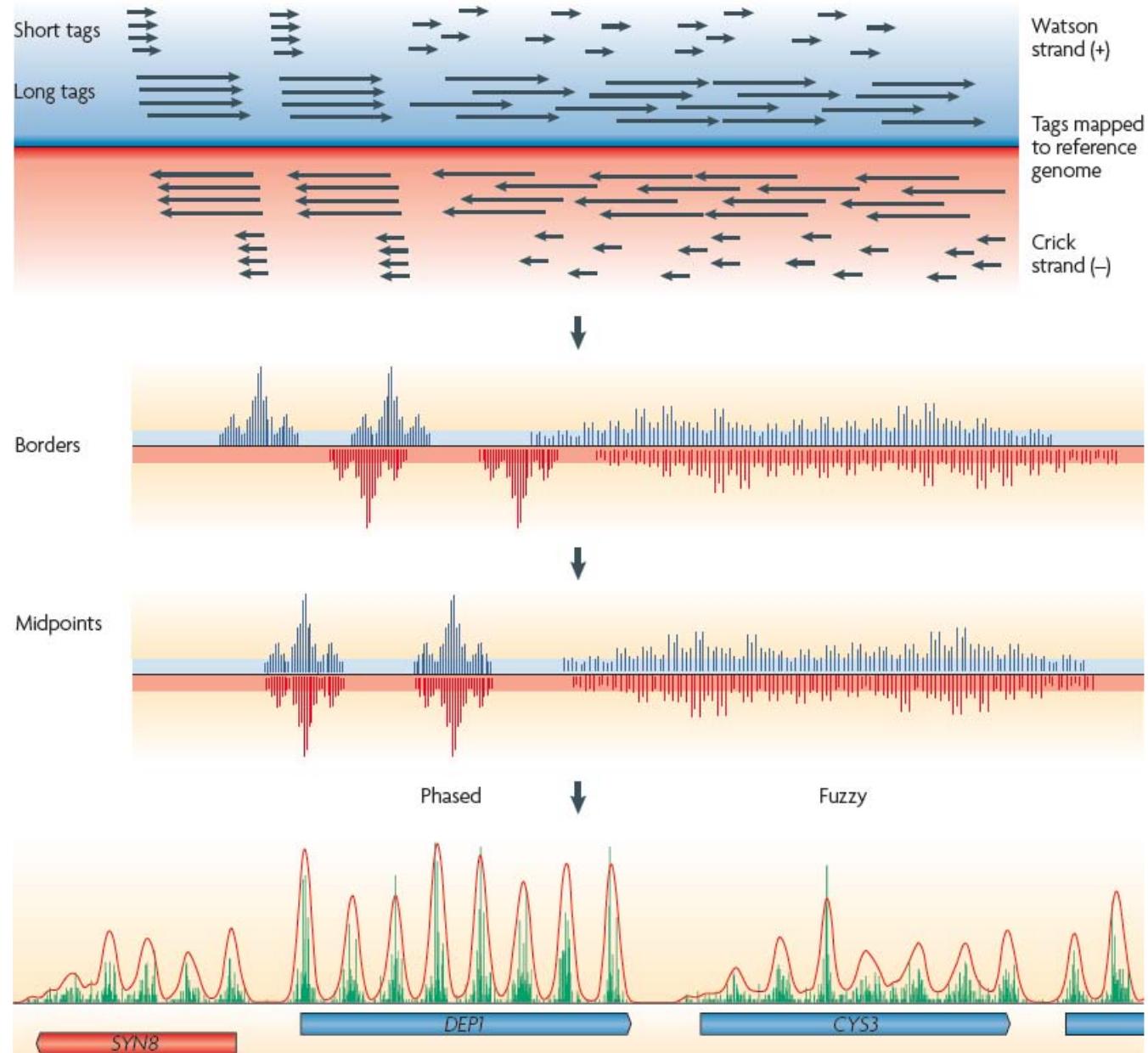
MNase-Seq

a

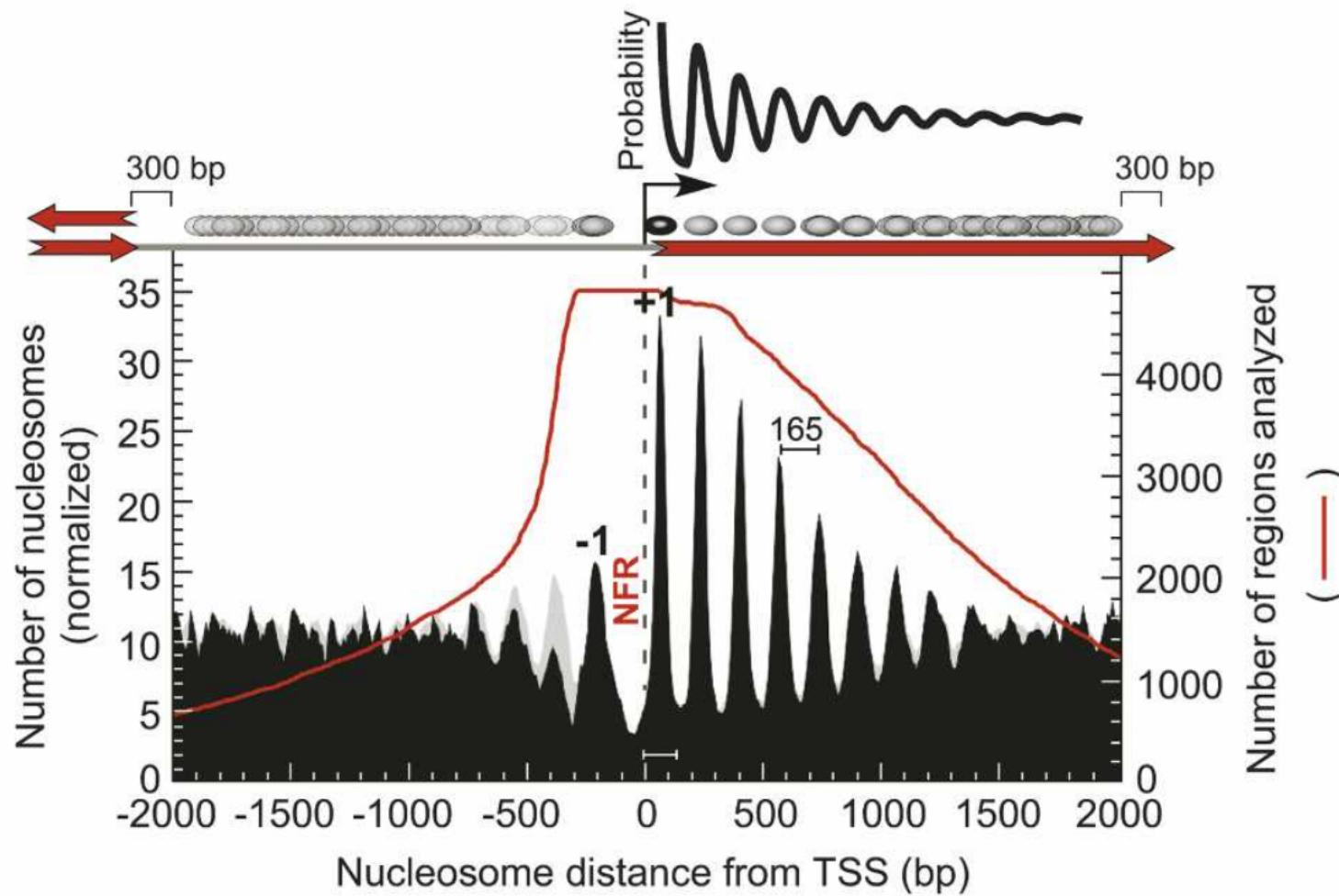


Mononucleosome signals

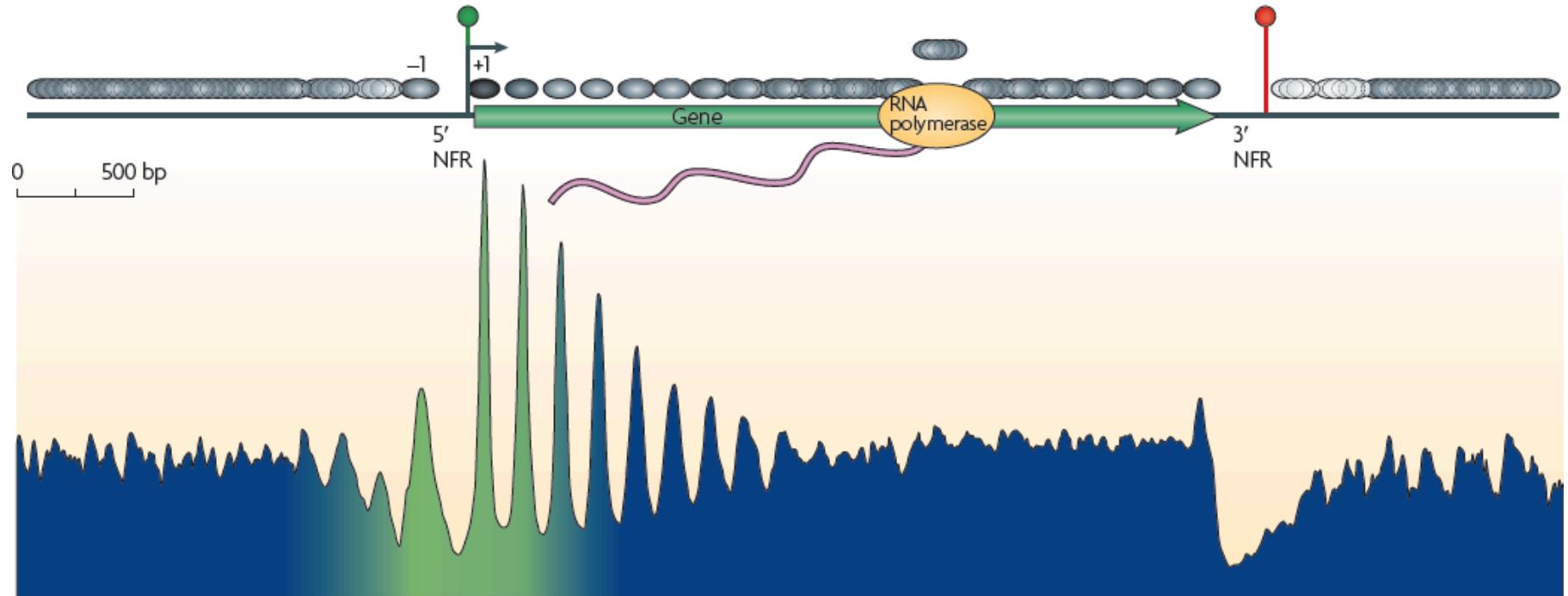
- Strand shift, followed by density estimation
- Direct detection of shifted strand patterns



Nucleosome positioning around TSS



Nucleosome positioning



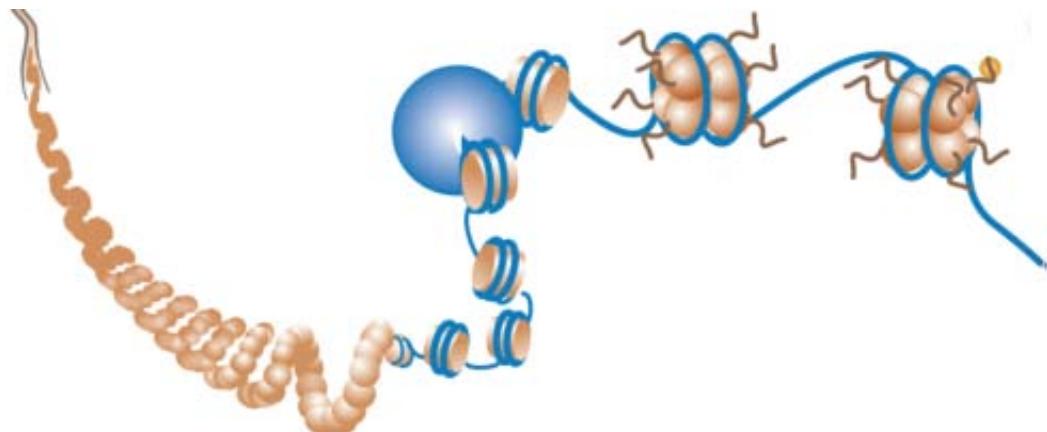
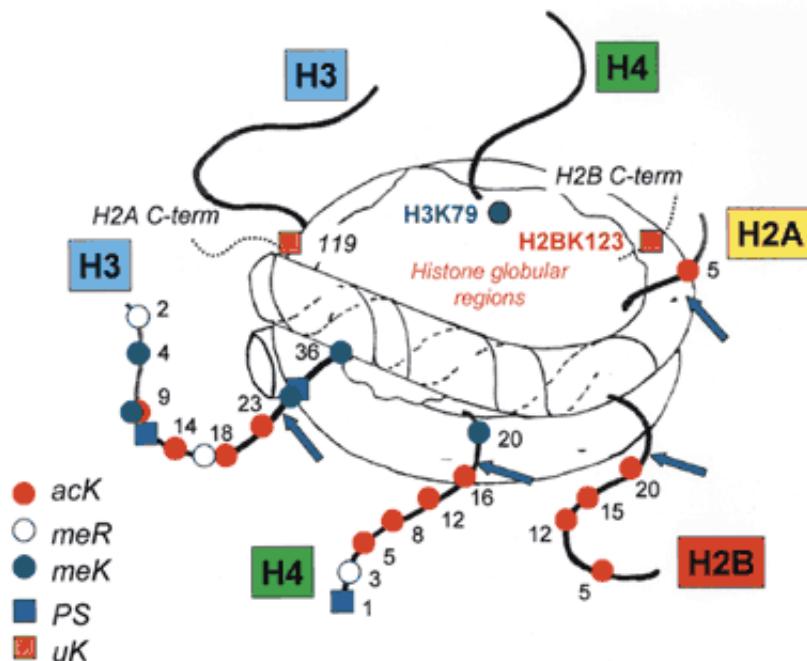
- Well-positioned nucleosomes; downstream of active TSS
- Nucleosome Free Regions (NFR) upstream of TSS
- Turnover, Variants and modifications

Epigenetics assays using NGS

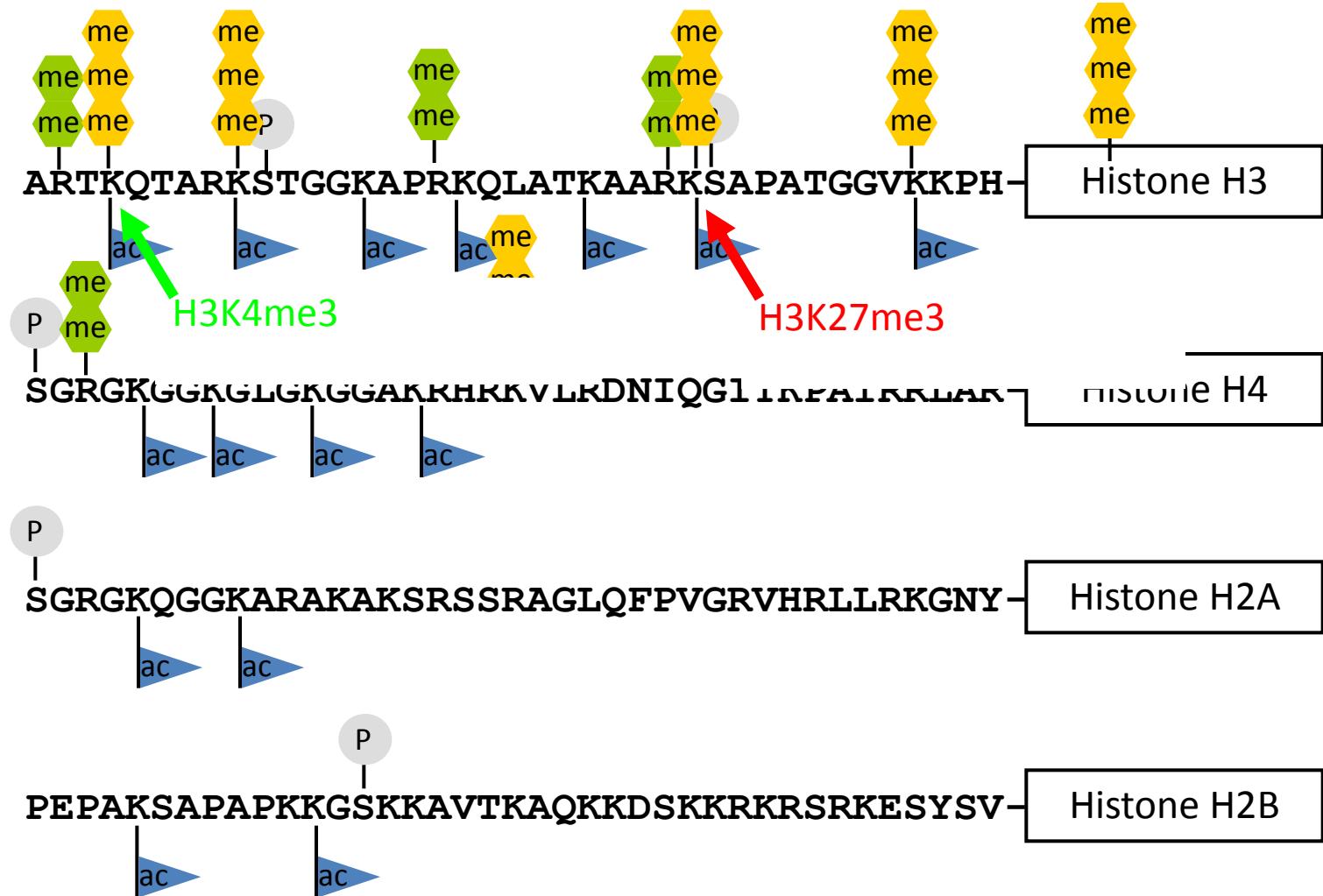
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Histone modifications and variations

- Covalent modifications
 - acetylation, methylation, ubiquitination, phosphorylation,
- Histone variants
 - H2A.Z, H3.3, H2A.X,
- Functional differences
- Enrich for particular type of nucleosomes
- Use specific antibodies
- **Chromatin Immunoprecipitation (ChIP)**

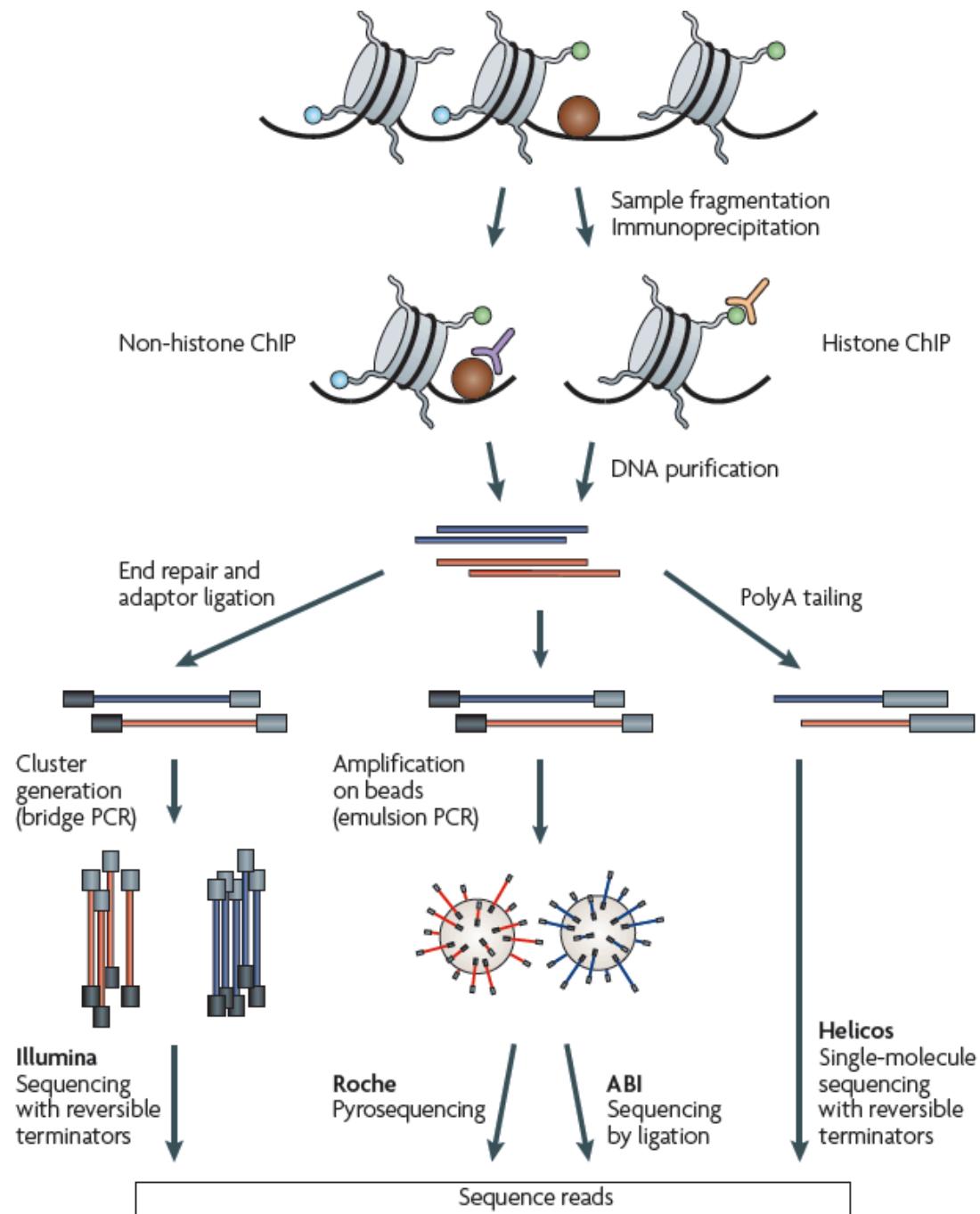


There are multiple histone modifications

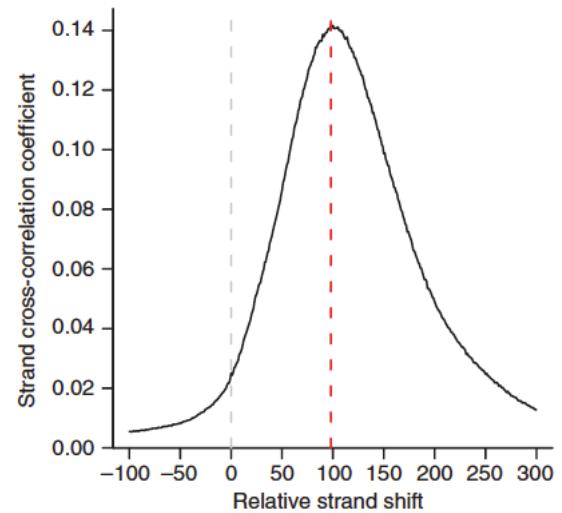
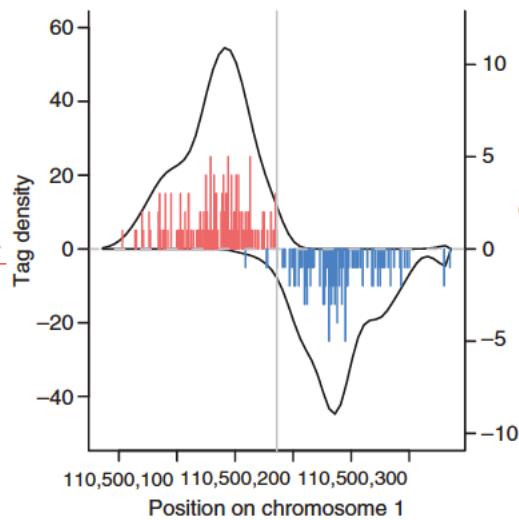
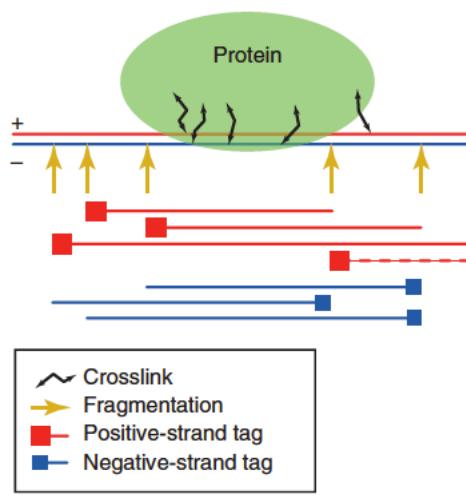


ChIP-seq

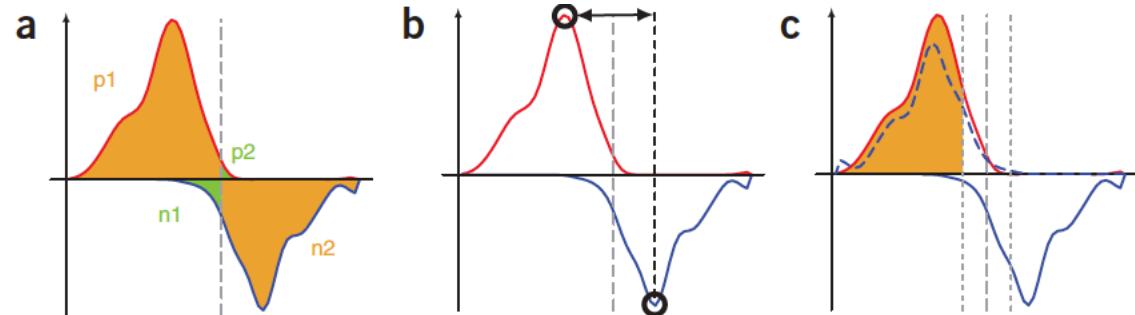
- DNA fragmentation
 - sonication
 - MNase
 - combinations
- Immunoprecipitation
 - Histone or DNA protein antibodies
 - Tags
- Crosslinking
- Amplification, library preparation



ChIP-seq : binding positions

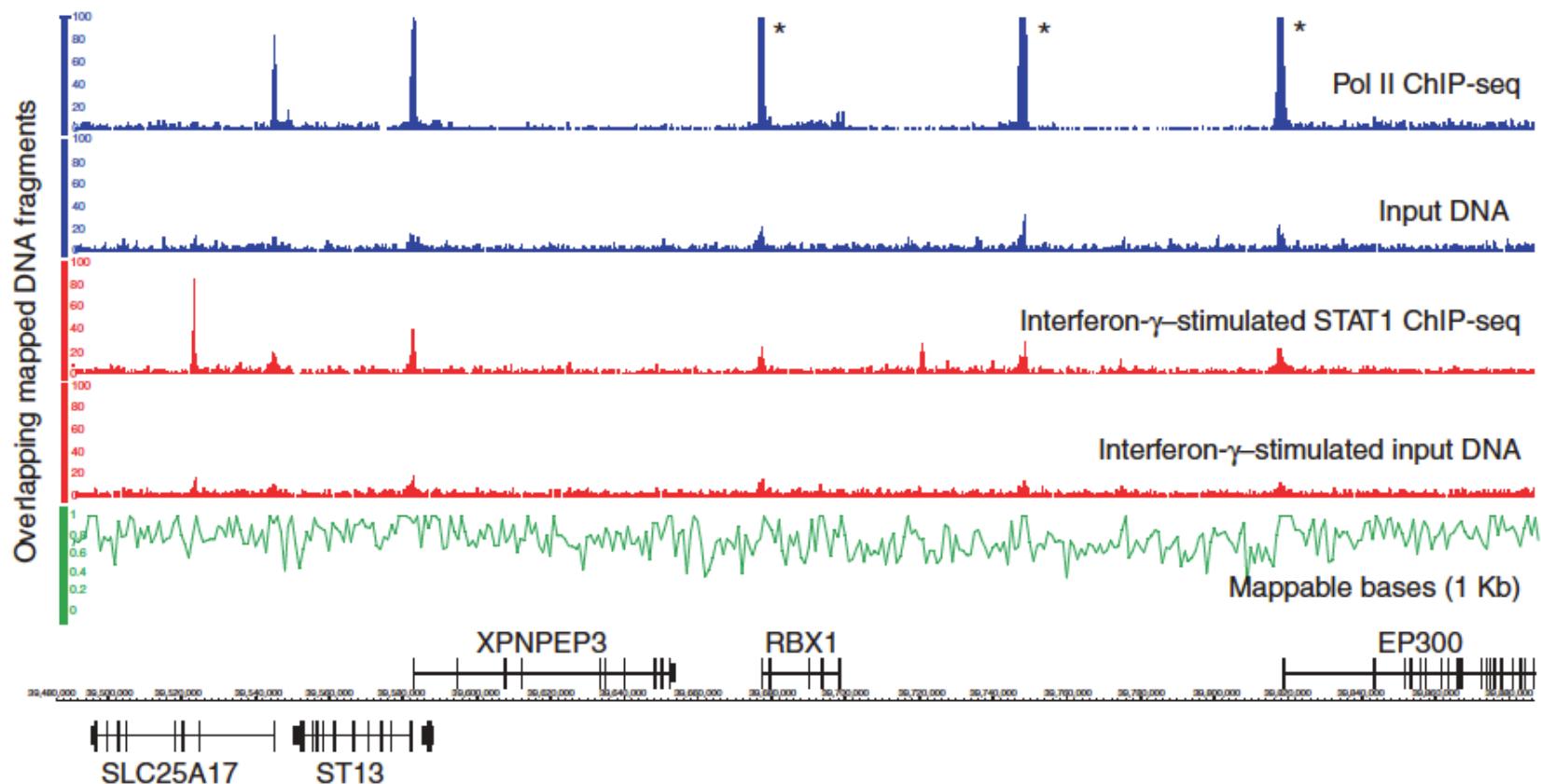


- Stranded pattern
- Peak detection methods
 - PeakSeq, MACS, SPP, QuEST



Assessing epitope enrichment

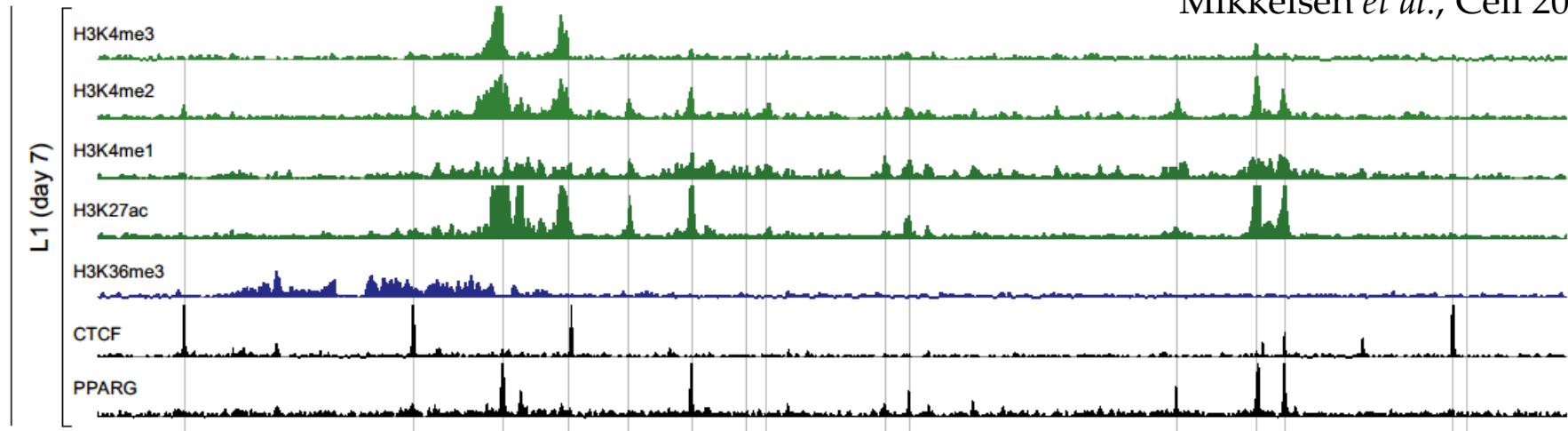
- Uneven background
 - seq. biases (fragmentation, GC, start)
 - mappability
- Controls
 - input chromatin
 - mock/non-specific Ab



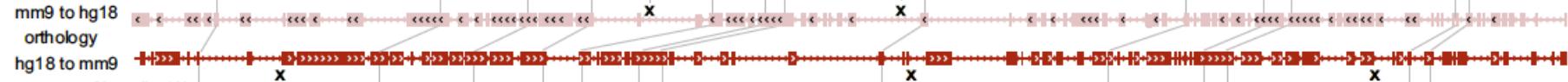
ChIP-seq example : adipogenesis

Mikkelsen *et al.*, Cell 2010

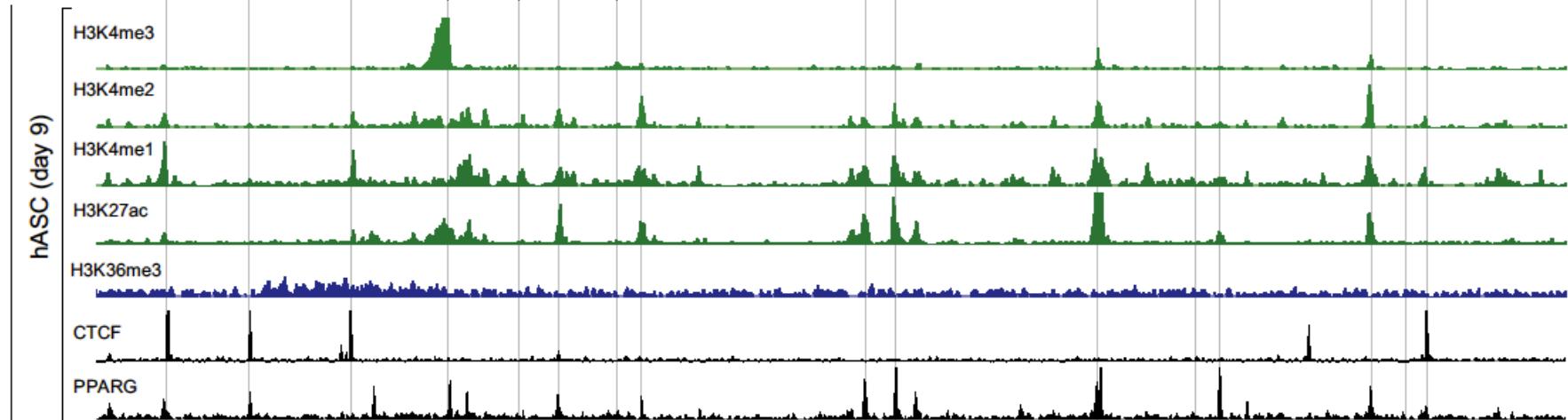
ChIP-Seq fragment densities



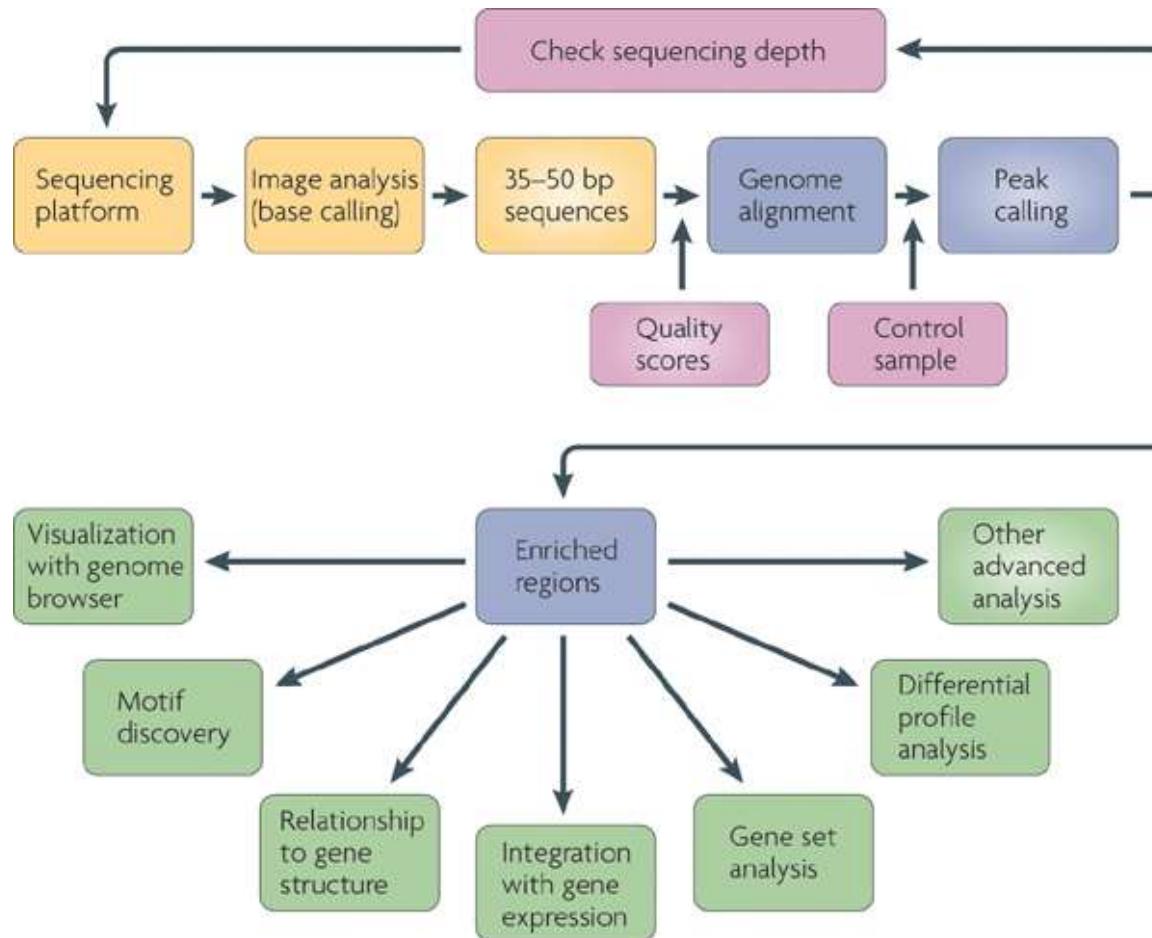
Chr 5 (mm9)



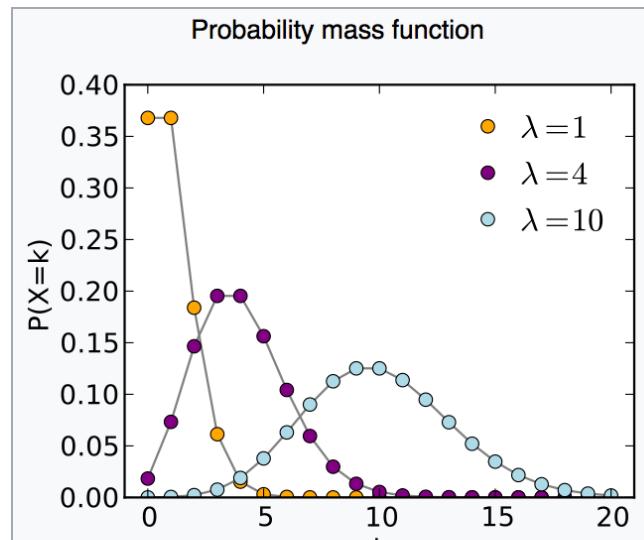
ChIP-Seq fragment densities



Pipeline for ChIP-seq data analysis



Number of reads from RNA-seq/ChIP-seq fit Poisson distribution



$$P(k \text{ events in interval}) = e^{-\lambda} \frac{\lambda^k}{k!}$$

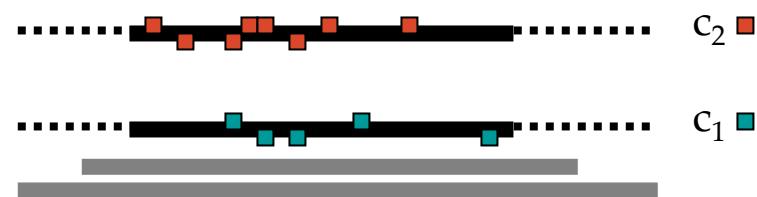
where

- λ is the average number of events per interval
- e is the number 2.71828... ([Euler's number](#)) the base of the natural logarithms
- k takes values 0, 1, 2, ...

ChIP-seq : estimating enrichment magnitude

- Log intensity ratios (M) : $\text{Log2}[\text{ChIP} / \text{input}]$
- Poisson process

- Sequencing depth instead of time
- Constant rate at a local scale
- Estimate ratio of two Poisson rates



- MLE $\hat{r} = \frac{\hat{\lambda}_1}{\hat{\lambda}_2} = R \frac{(c_1 + 0.5)}{(c_2 + 0.5)}$ $R = \frac{S_2}{S_1}$
- Bounds $r^{(1-\alpha)} = R \frac{(c_1 + 0.5)}{(c_2 + 0.5)} F_{1-\alpha, 2(c_1+0.5), 2(c_2+0.5)}$

- Sliding windows,
conservative estimates,
broad regions of enrichment
- Spatial scale

- Testing for enrichment
 - binomial model
 - *background (R)* corrections

SICER for calling Histone peaks

1. Remove duplicate reads

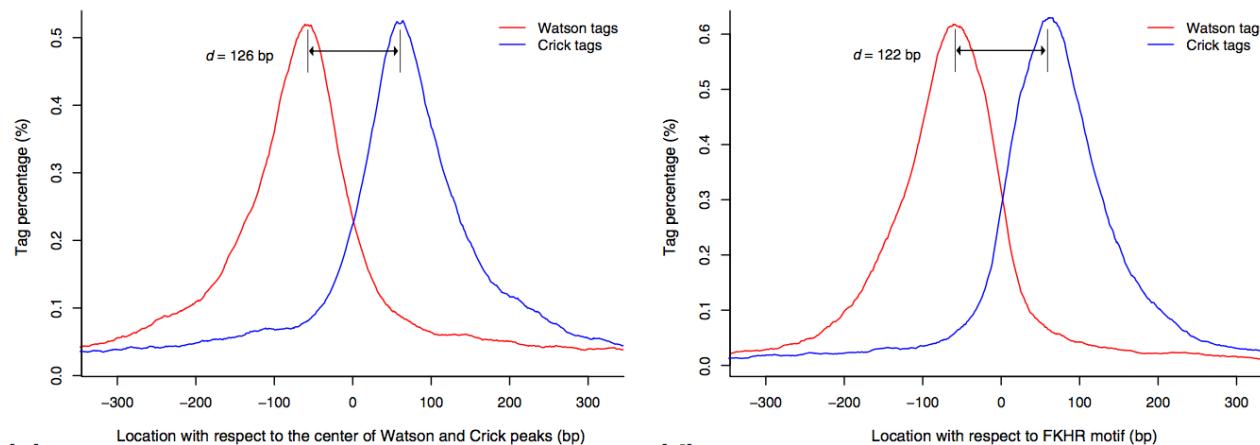
2.1 The island approach

2.1.1 Scoring scheme We partition the genome of effective length L into non-overlapping windows of size w . We define the score s for a window with l reads to be $s(l) = -\log P(l, \lambda)$. $P(l, \lambda)$ is a Poisson distribution parameterized by the average number of reads in a window $\lambda = wN/L$, where N is the total number of reads in the ChIP-Seq library. Given this definition, the scores

MACS for calling TF peaks

1. Remove duplicate reads

2. Modeling the shift size of ChIP-Seq tags



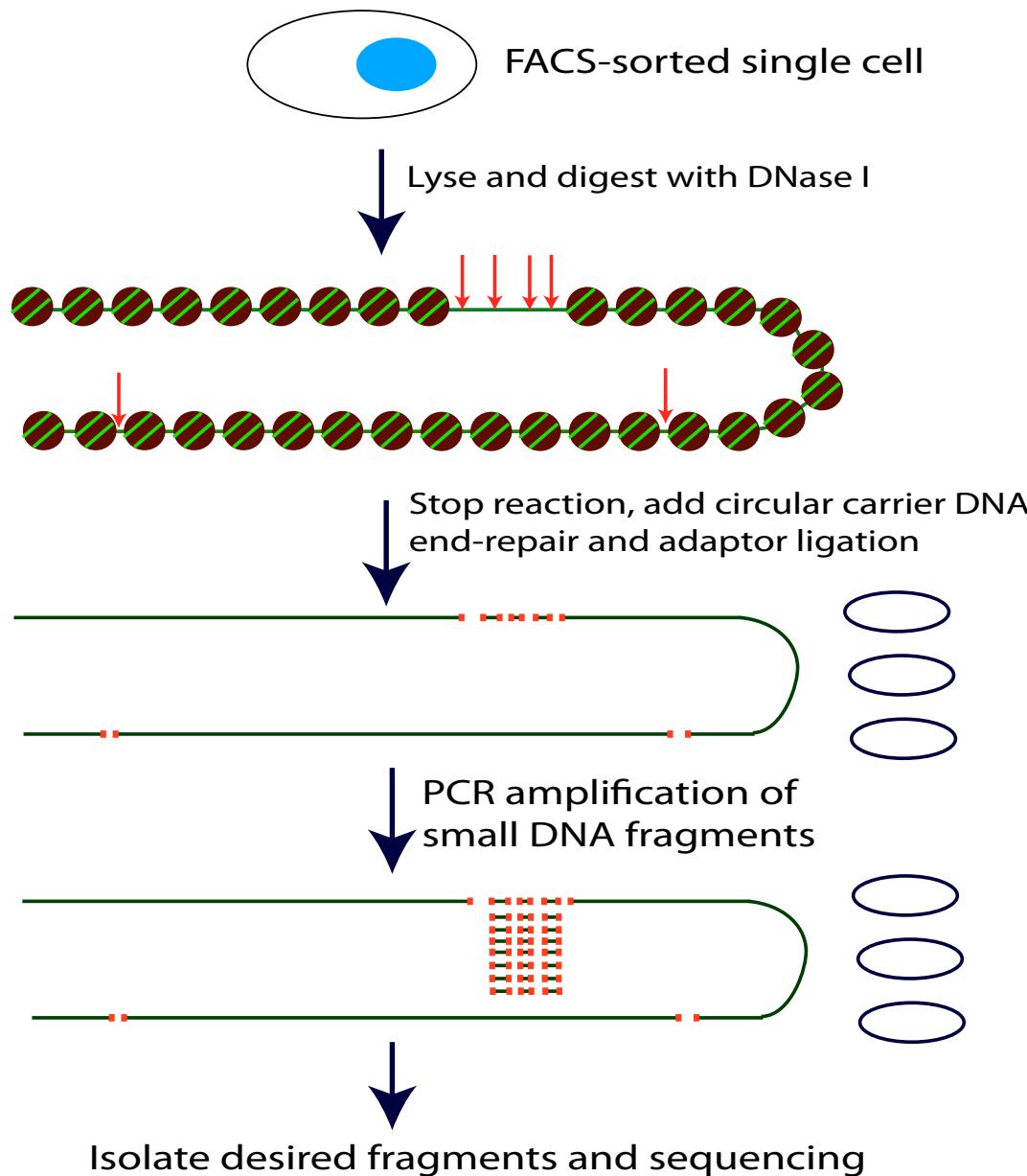
3. Peak detection based on local reads distribution

$$\lambda_{\text{local}} = \max(\lambda_{\text{BG}}, [\lambda_{1k}, \lambda_{5k}, \lambda_{10k}])$$

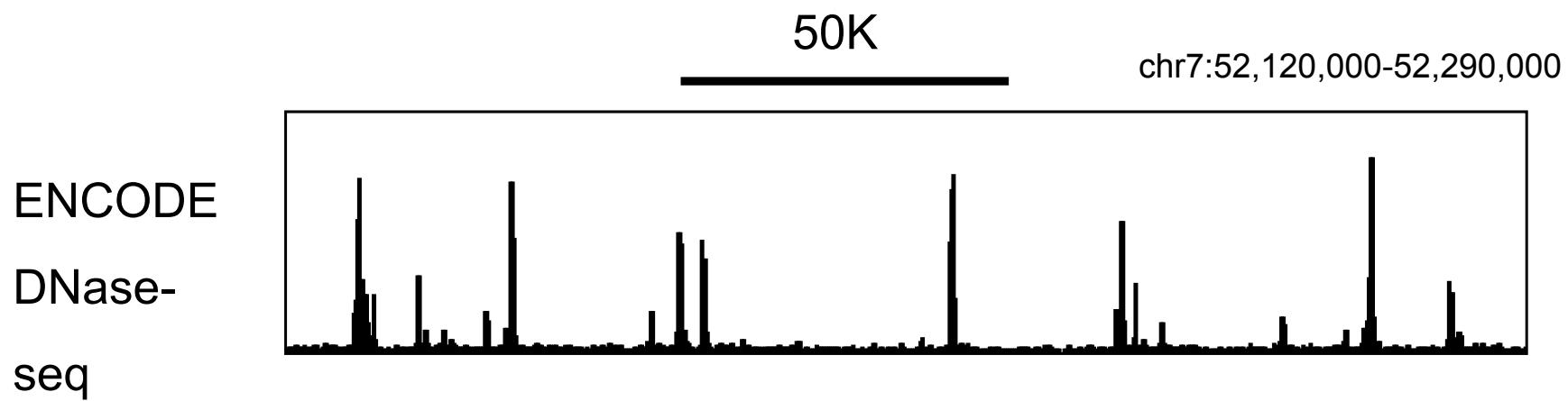
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Schema of DNase-Seq



DNase-seq is a powerful tool for genome-wide mapping of regulatory elements



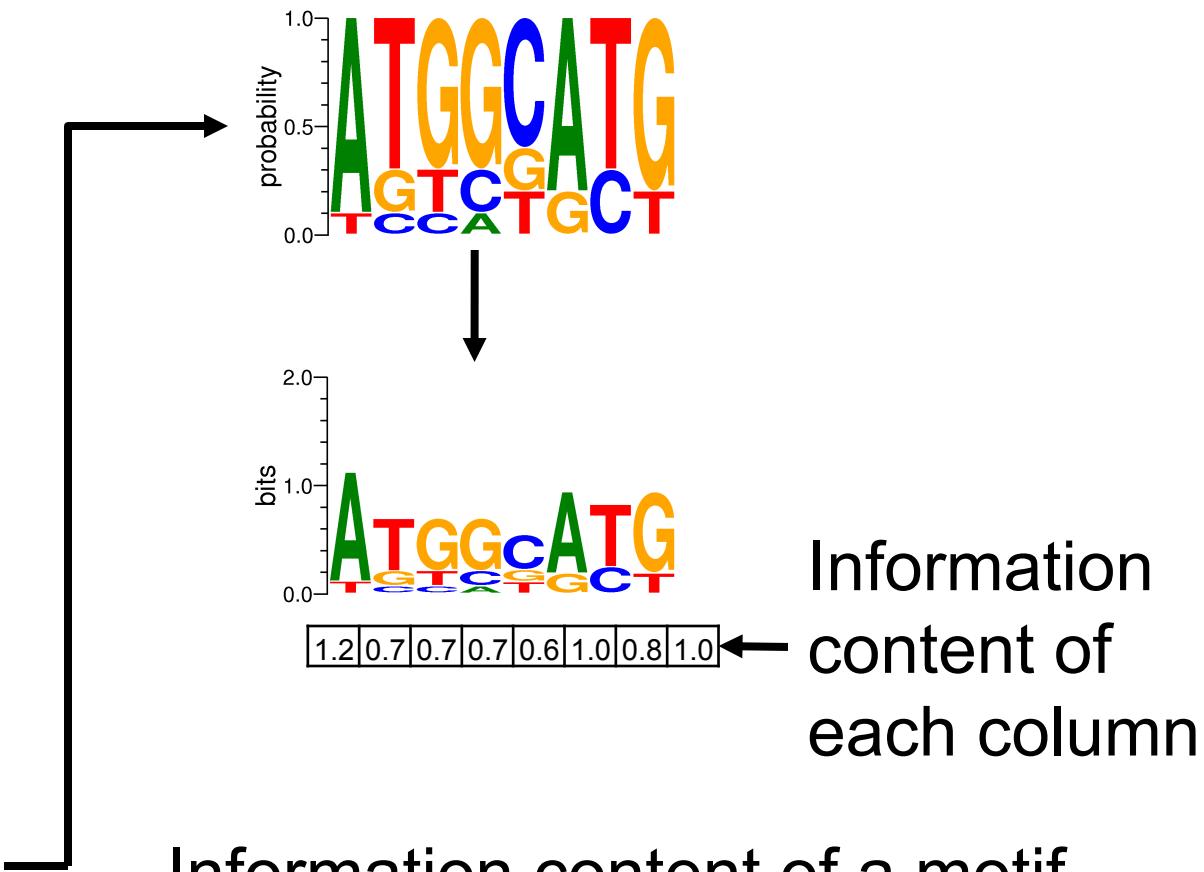
Boyle, *et al. Cell*, 2008

Recall: TFBS Position Weight Matrix (PWM)

Experimentally determined sites									
A	T	G	G	C	A	T	G		
A	G	G	G	T	G	C	G		
A	T	C	G	C	A	T	G		
T	T	G	C	C	A	C	G		
A	T	G	G	T	A	T	T		
A	T	T	C	G	A	C	G		
A	G	G	G	C	G	T	T		
A	T	G	A	C	A	T	G		
A	T	G	G	C	A	T	G		
A	C	T	G	G	A	T	G		

Alignment (count) Matrix									
A	9	0	0	1	0	8	0	0	
C	0	1	1	1	7	0	3	0	
G	0	2	7	8	1	2	0	8	
T	1	7	2	0	2	0	7	2	

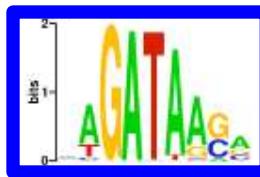
Frequency Weight Matrix									
A	0.9	0.0	0.0	0.1	0.0	0.8	0.0	0.0	
C	0.0	0.1	0.1	0.1	0.7	0.0	0.3	0.0	
G	0.0	0.2	0.7	0.8	0.1	0.2	0	0.8	
T	0.1	0.7	0.2	0.0	0.2	0.0	0.7	0.2	
Cons	A	T	G	G	C	A	T	G	



Information content of a motif
= sum of all columns
= $1.2 + 0.7 + 0.7 + 0.6 + 1.0 + 0.8 + 1.0 = 6.0$

Motif discovery and enrichment analysis

Th2



 GATA motif

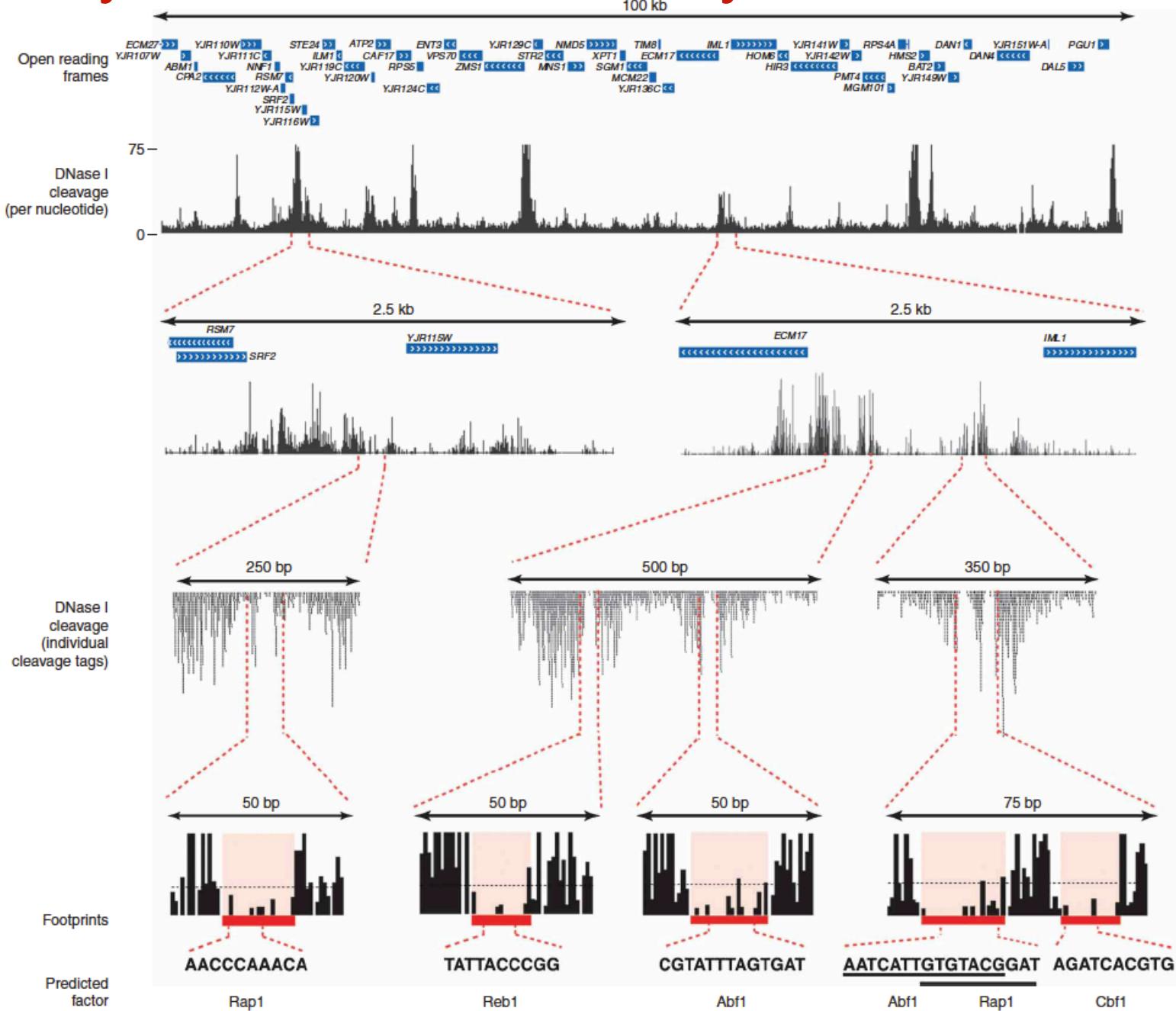
 AP-1 motif

 Ets motif

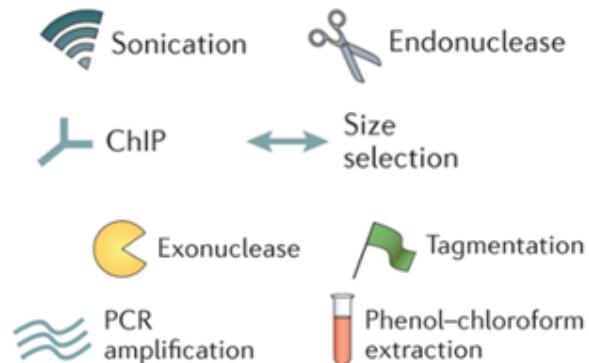
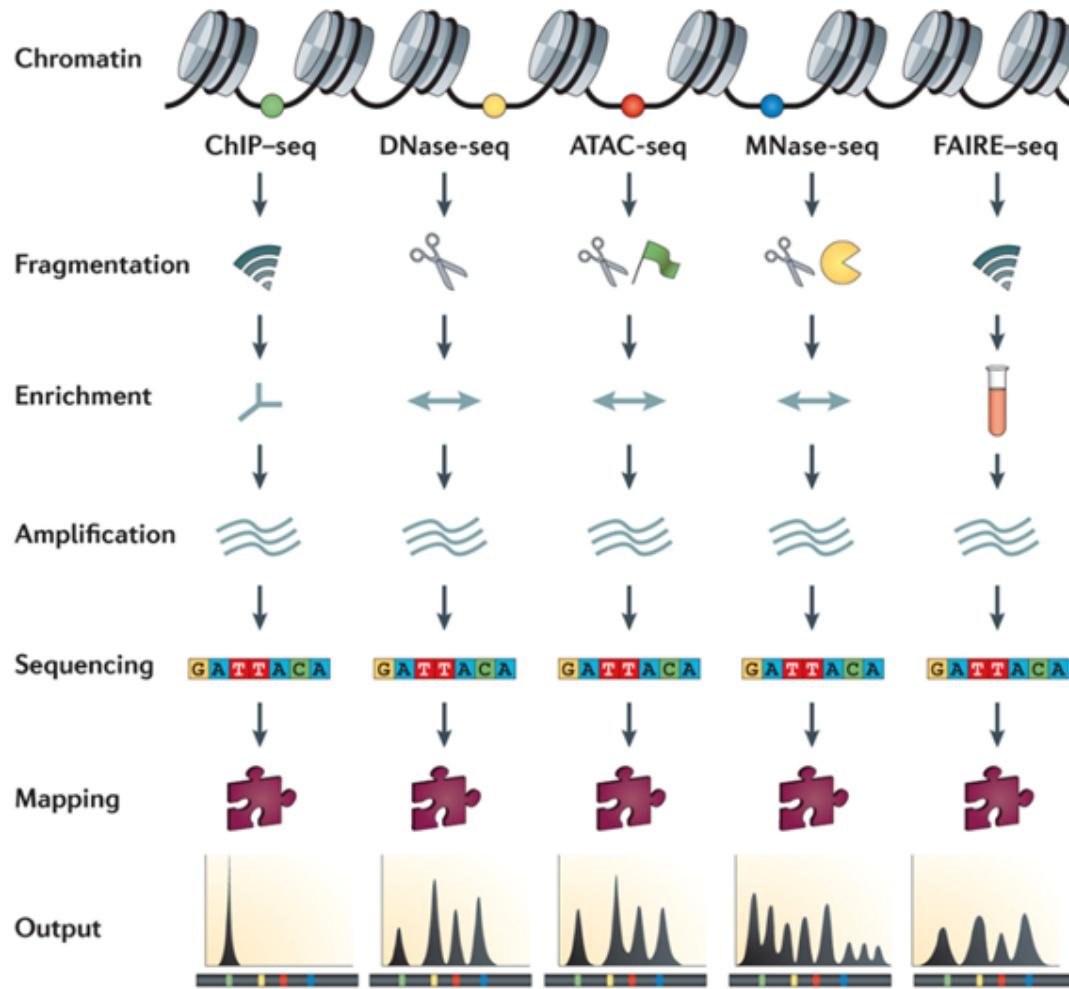
 Runx motif

Assays : DNase I sensitivity

Hesselberth *et al.*, Nat. Methods 2009



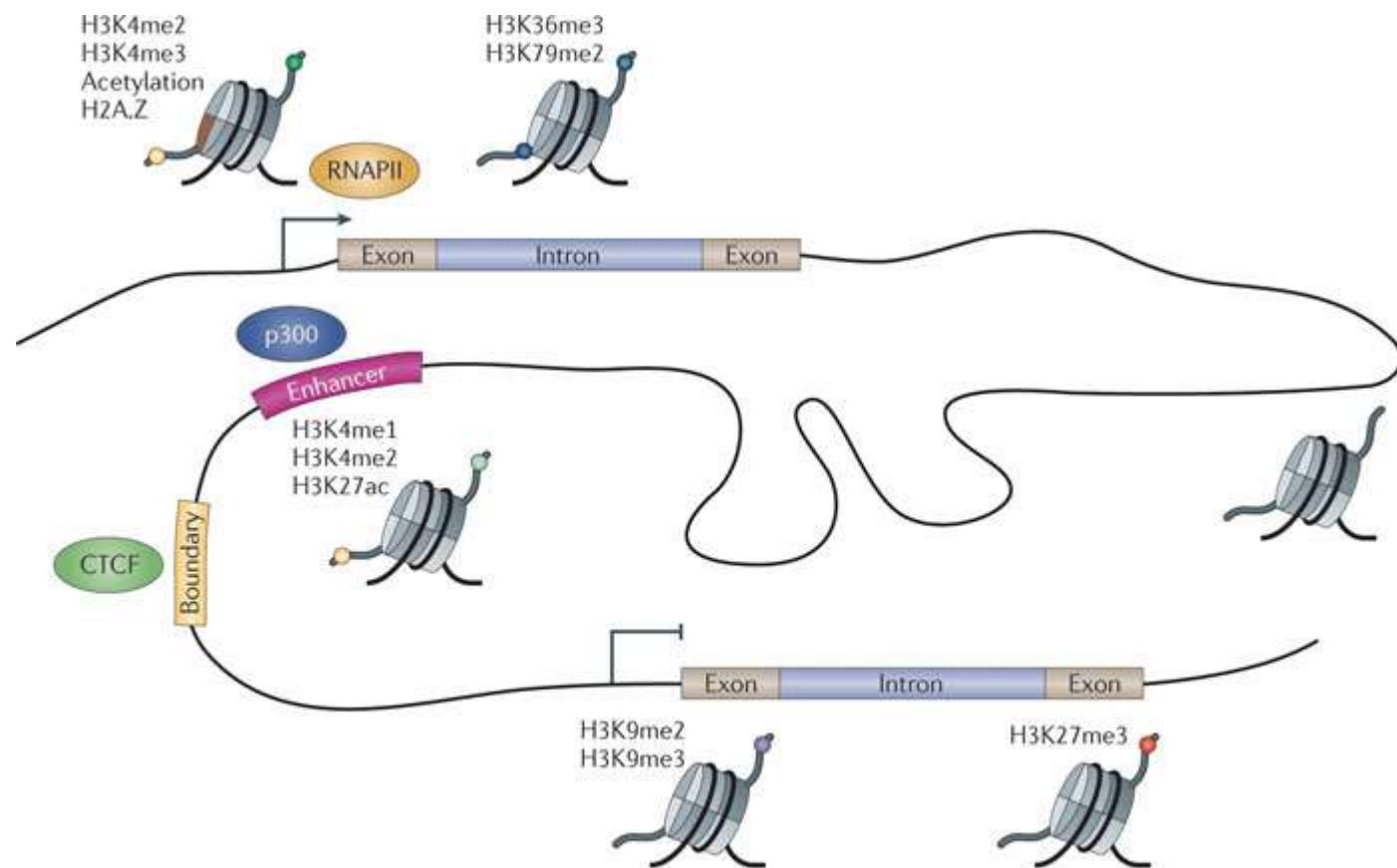
Summary of epigenetic locus study



Epigenetics assays using NGS

- Bisulfite-Sequencing (BS-seq)
 - DNA methylation
- MNase-seq
 - Nucleosome positioning
- Chromatin Immunoprecipitation sequencing (ChIP-seq)
 - Signatures of protein association
 - Histone variants and histone modification
- DNase-seq, ATAC-seq
 - Chromatin accessibility
- Hi-C, CHIA-PET
 - Chromatin architecture

Regulatory function of chromatin interactions



Chromosome Conformation Capture (3C)

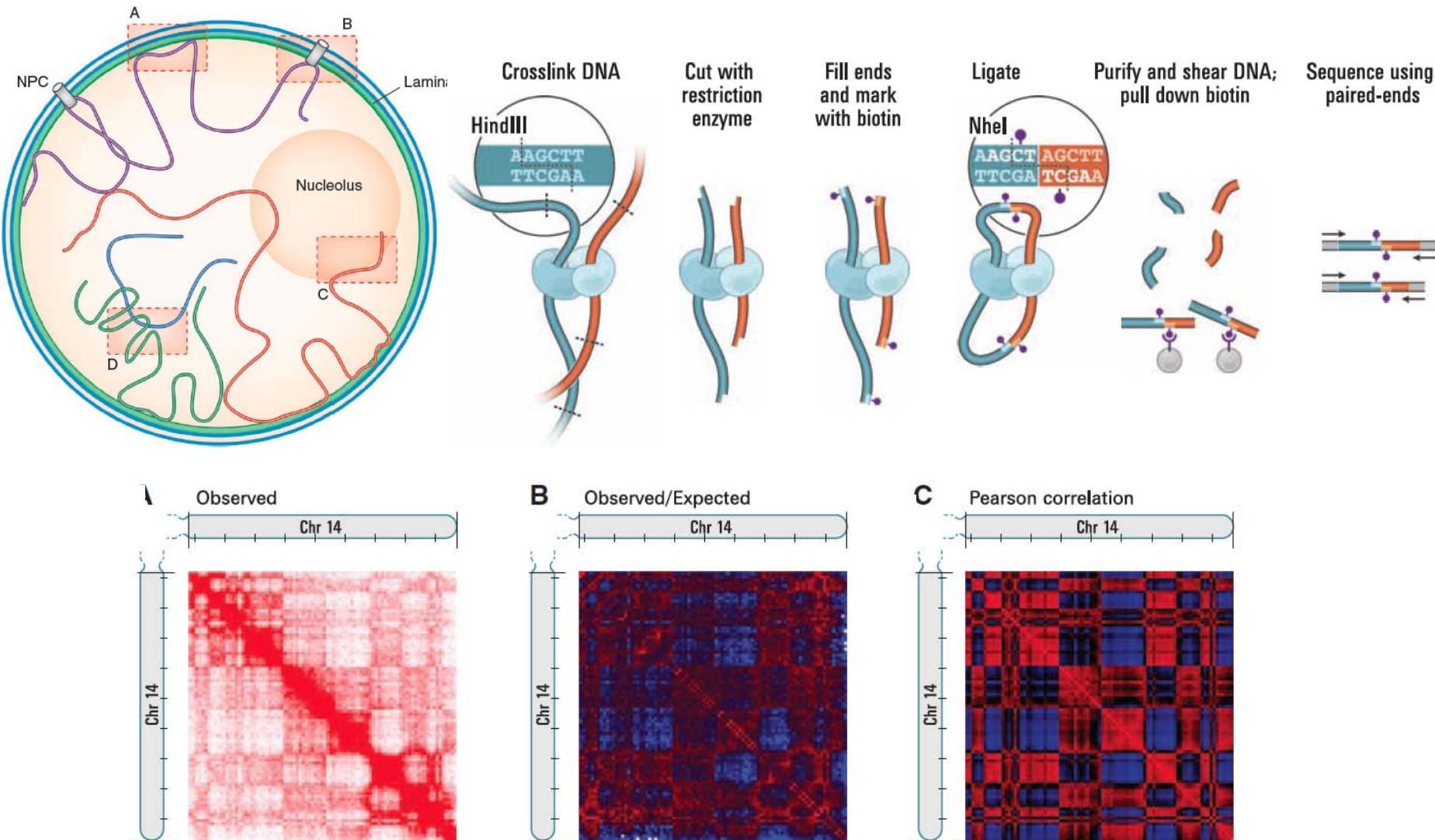
a 3C: converting chromatin interactions into ligation products



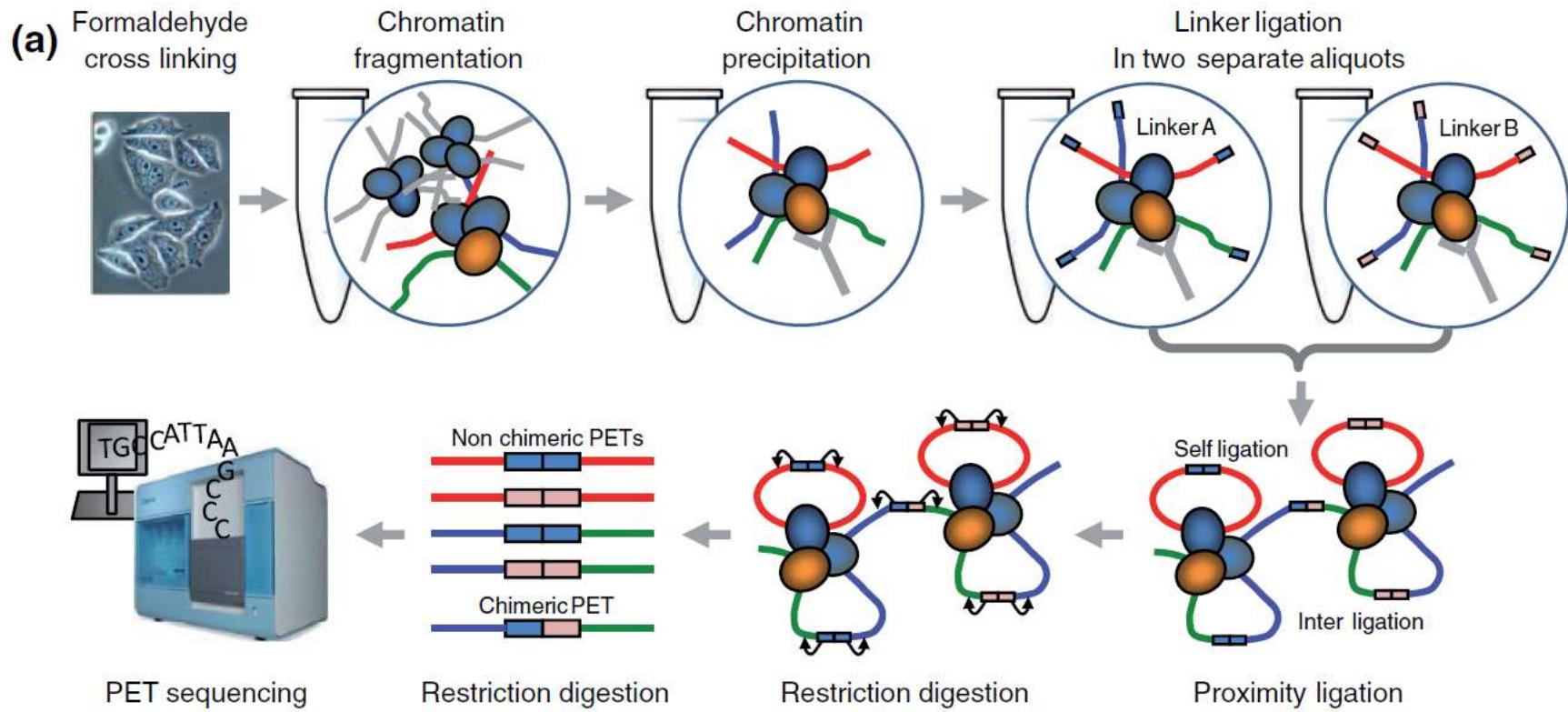
b Ligation product detection methods

3C	4C	5C	ChIA-PET	Hi-C
One-by-one All-by-all	One-by-all	Many-by-many	Many-by-many	All-by-all
			<ul style="list-style-type: none">• DNA shearing• Immunoprecipitation	<ul style="list-style-type: none">• Biotin labelling of ends• DNA shearing
PCR or sequencing	Inverse PCR sequencing	Multiplexed LMA sequencing	Sequencing	Sequencing

Assays: 3D genome architecture

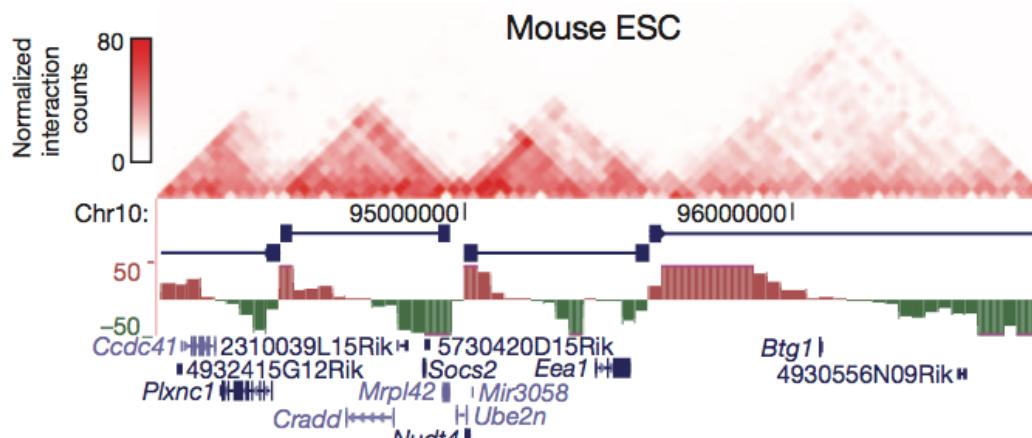


ChIA-PET protocol

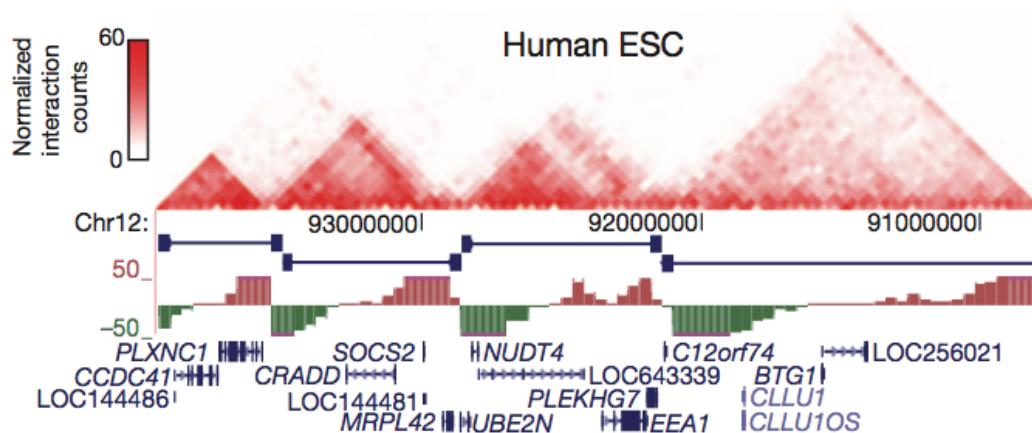


Topological associated domain (TAD)

g



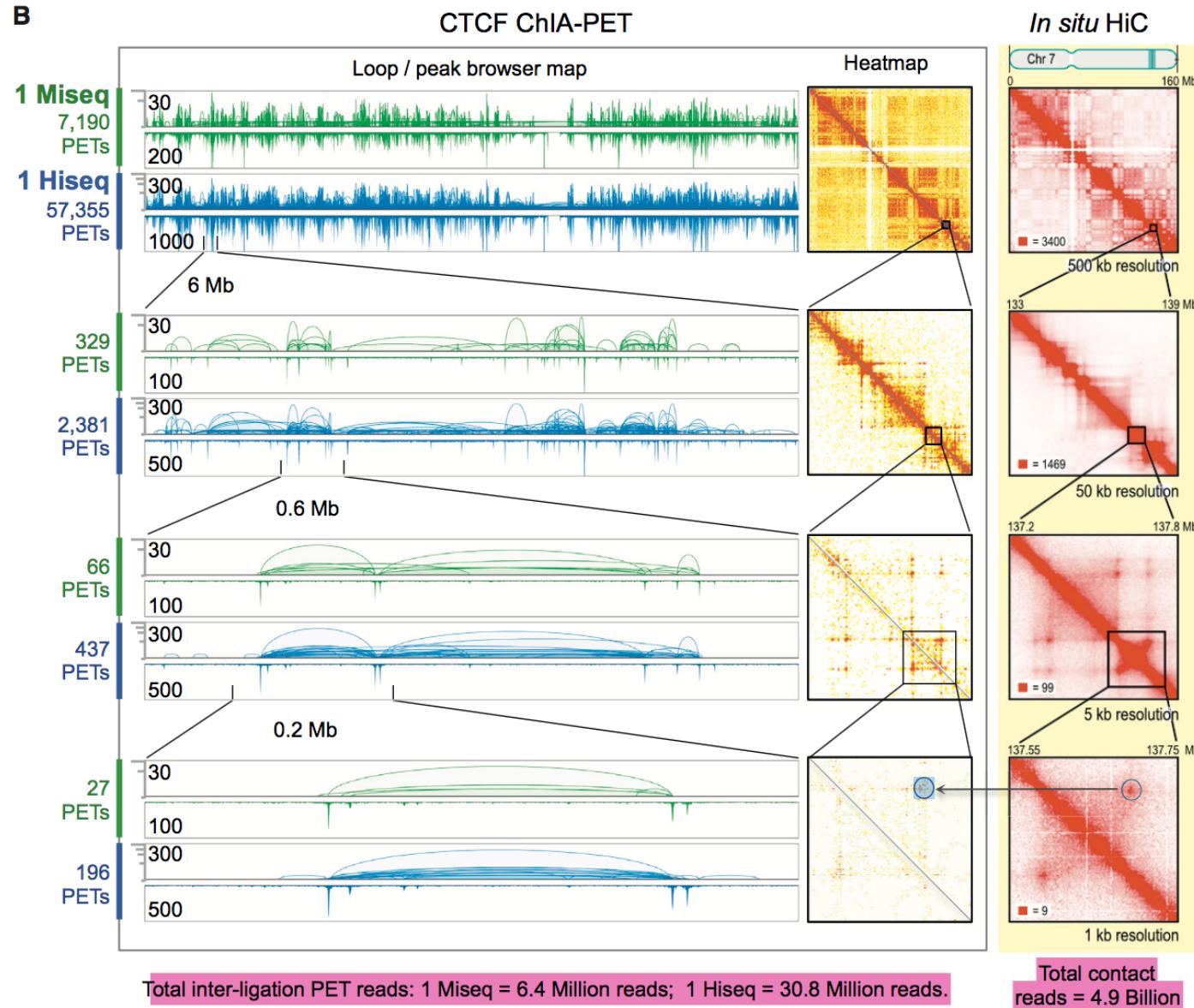
h



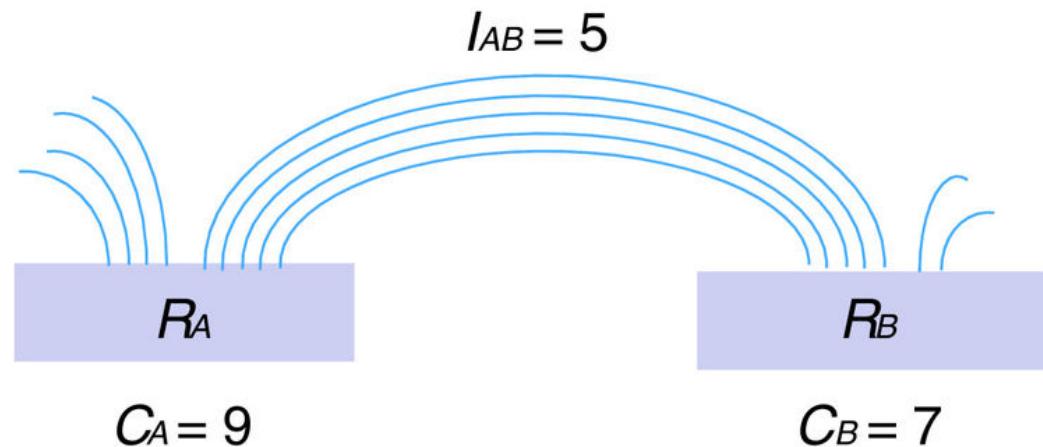
100Kb resolution

Dixon et al. 2012 Nature

Identification of chromatin loops



Identifying significant interaction



$$\Pr(I_{A,B} \mid N, c_A, c_B) = \frac{\binom{c_A}{I_{A,B}} \binom{2N - c_A}{c_B - I_{A,B}}}{\binom{2N}{c_B}}$$

Thank you for your attention