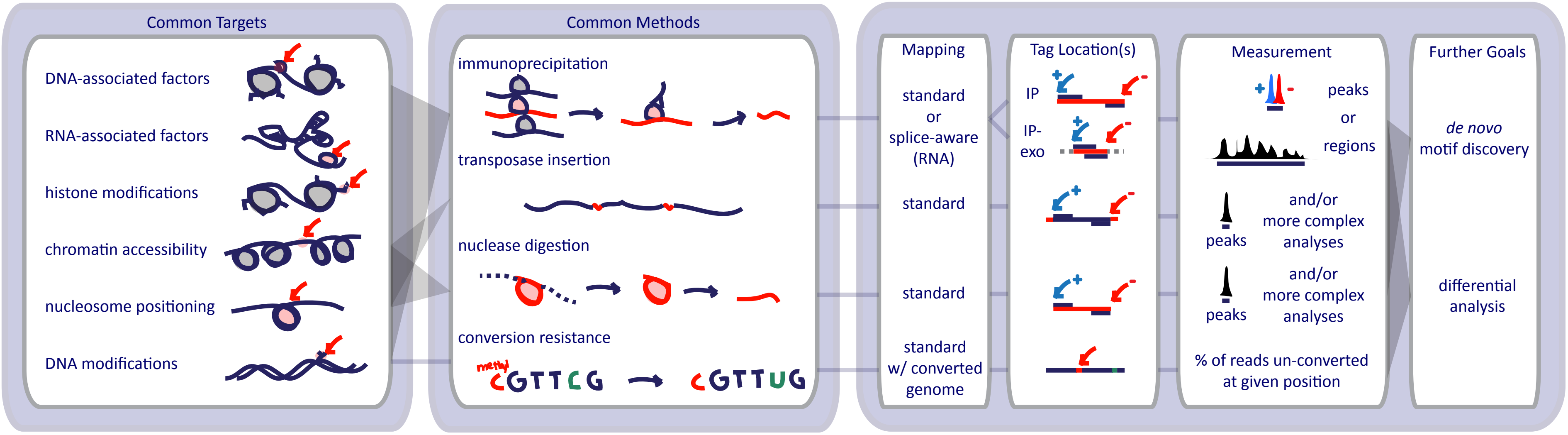
Epigenomics overview

BMI206 November 3, 2014



Common Targets

- DNA-associated factors
- RNA-associated factors
- histone modifications
- chromatin accessibility
- nucleosome positioning
- DNA modifications

Common Methods

- immunoprecipitation
- transposase insertion
- nuclease digestion
- conversion resistance

Mapping	Tag Location(s)	Measurement	Further Goals
standard or splice-aware (RNA)	IP IP-exo	peaks or regions	*de novo* motif discovery
standard		peaks and/or more complex analyses	
standard		peaks and/or more complex analyses	differential analysis
standard w/ converted genome		% of reads un-converted at given position	

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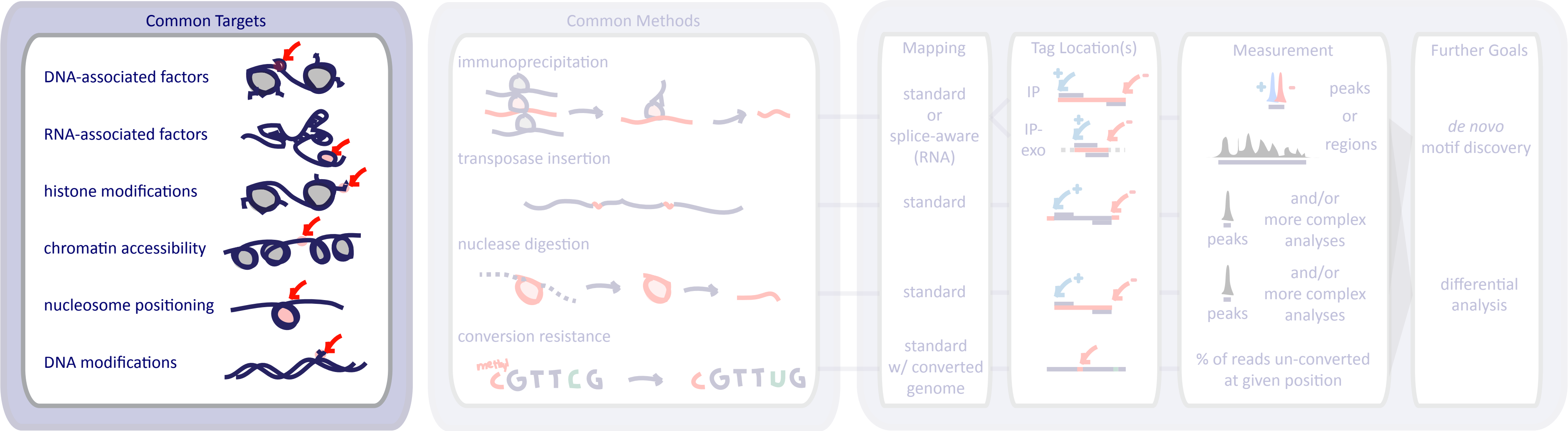

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- % of reads un-converted at given position

Further Goals

- *de novo* motif discovery
- differential analysis

Epigenomics overview

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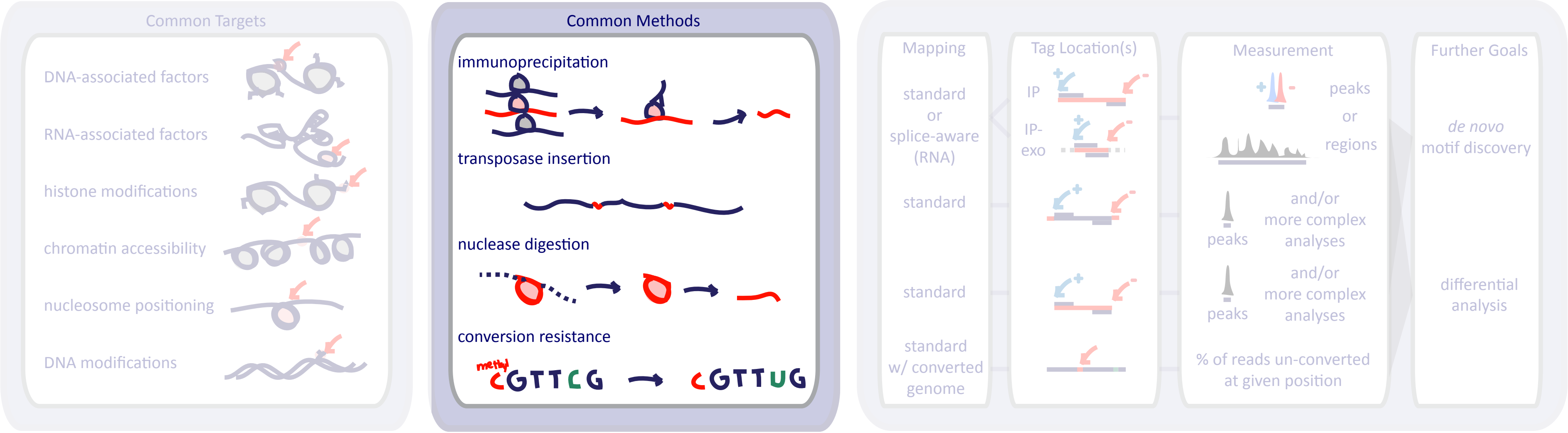

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DNA-associated factors

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nucleosome positioning

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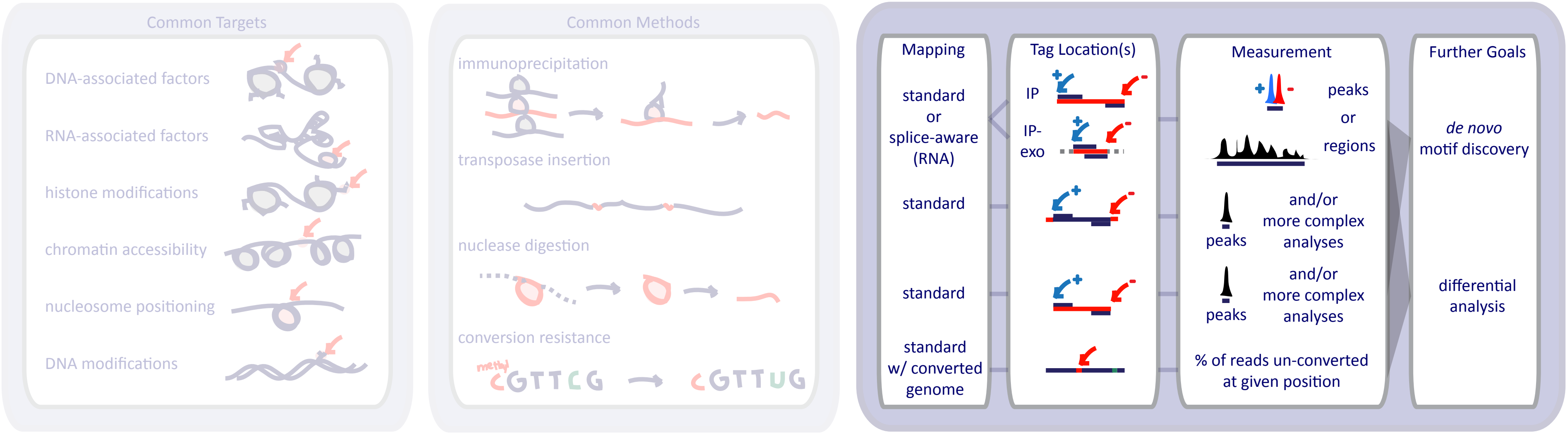

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Further Goals

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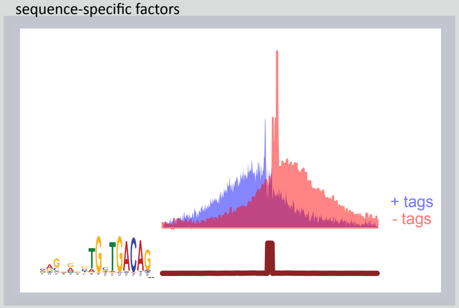
differential analysis

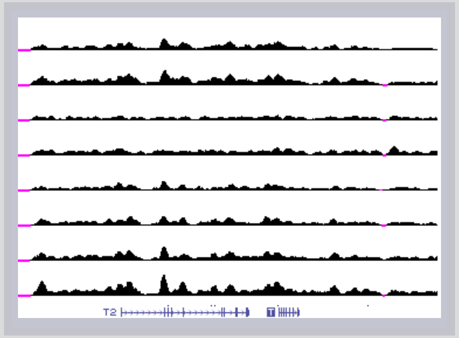
BMI206 November 3, 2014

DNA-associated factors

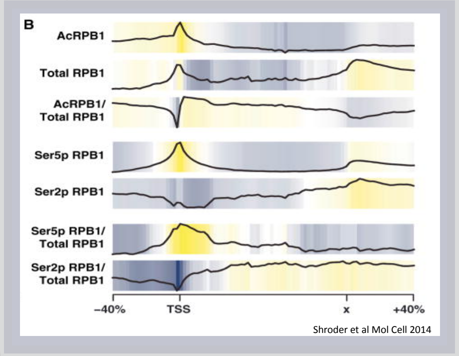
sequence-specific factors



chromatin associated



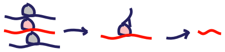
transcription machinery



Shroder et al Mol Cell 2014

methodology

immunoprecipitation

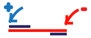


library construction

sequencing

mapping to genome reference (BWA or bowtie2)

identify tag locations



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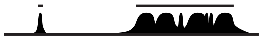

histone modifications

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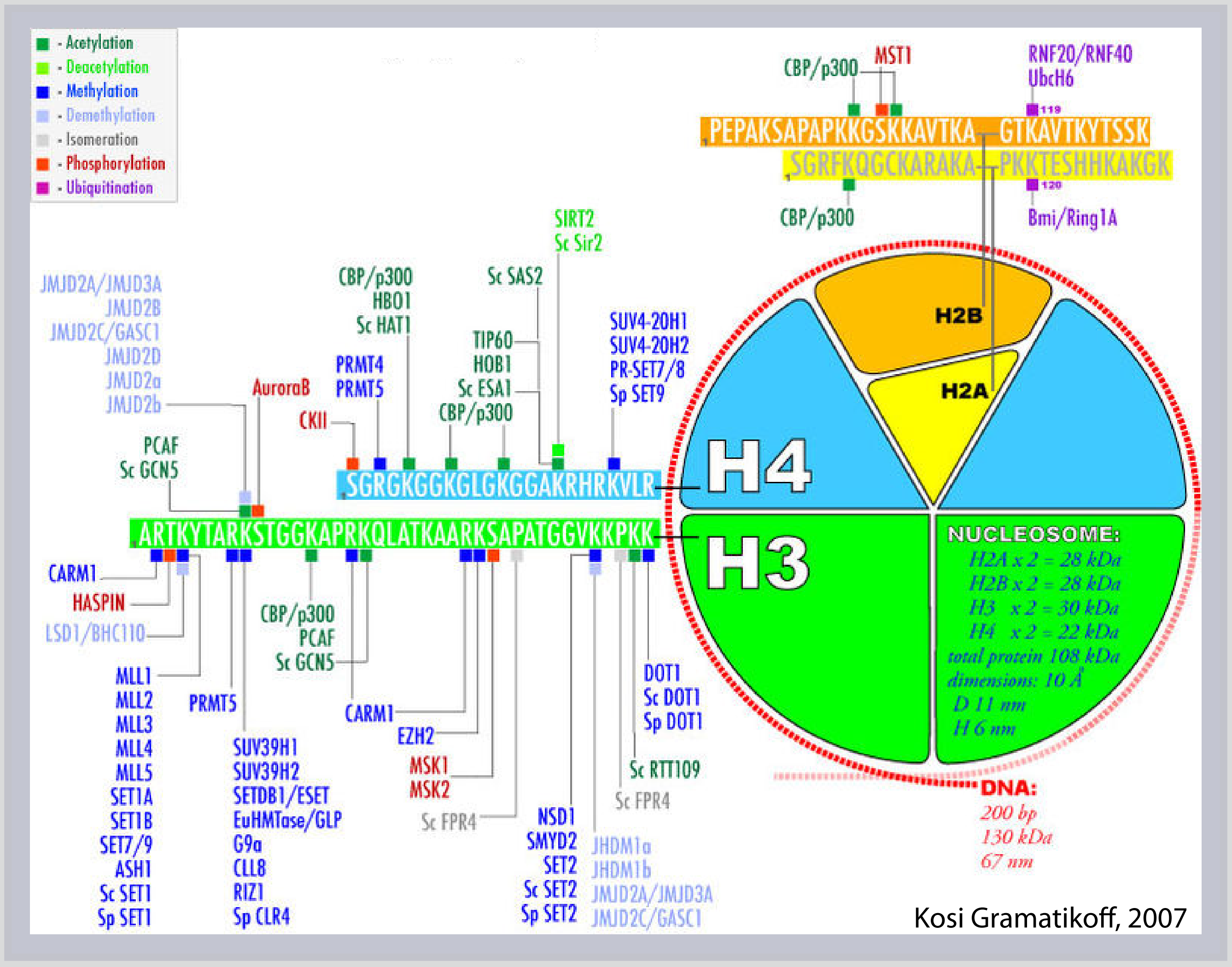
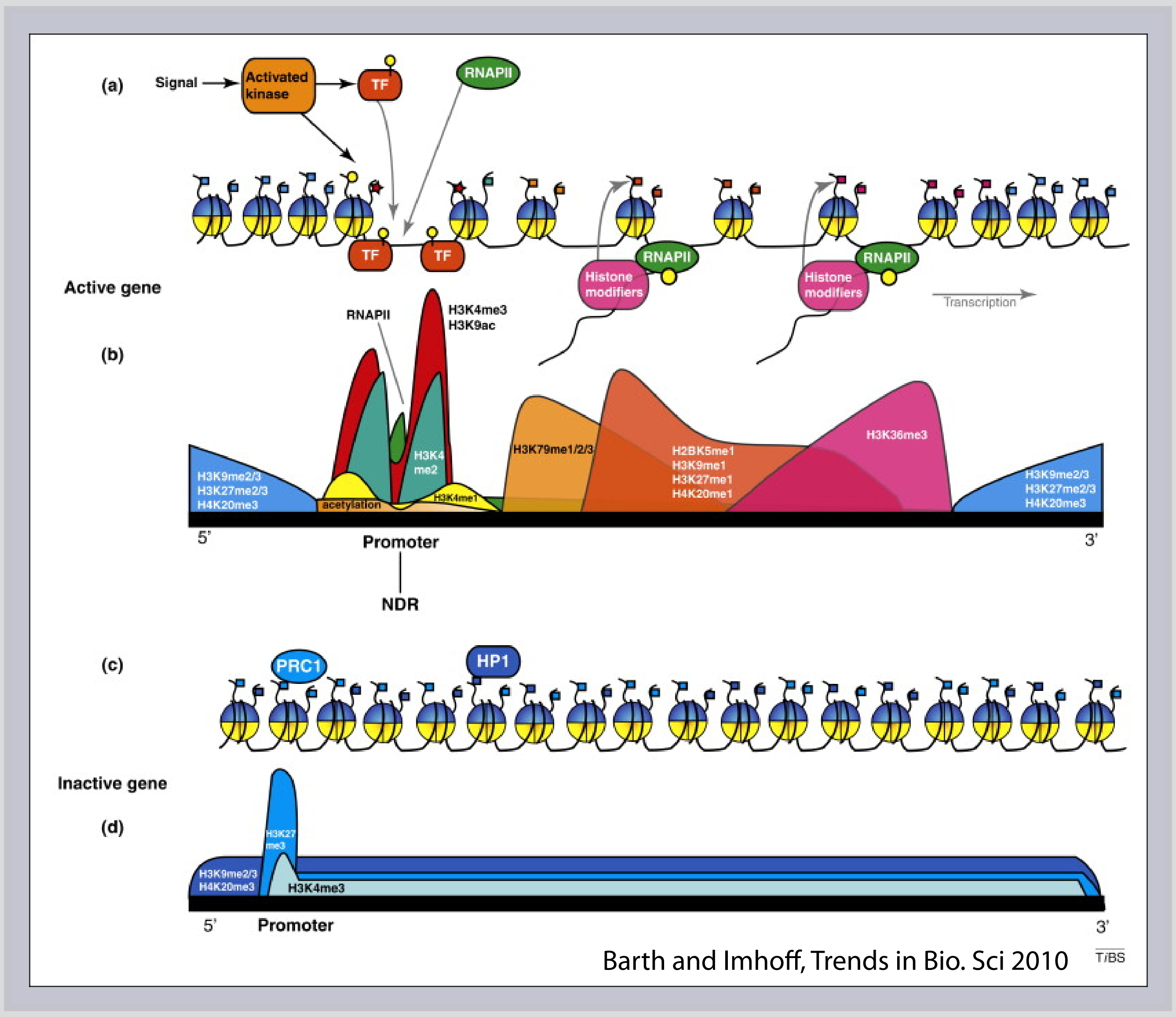
catalogue



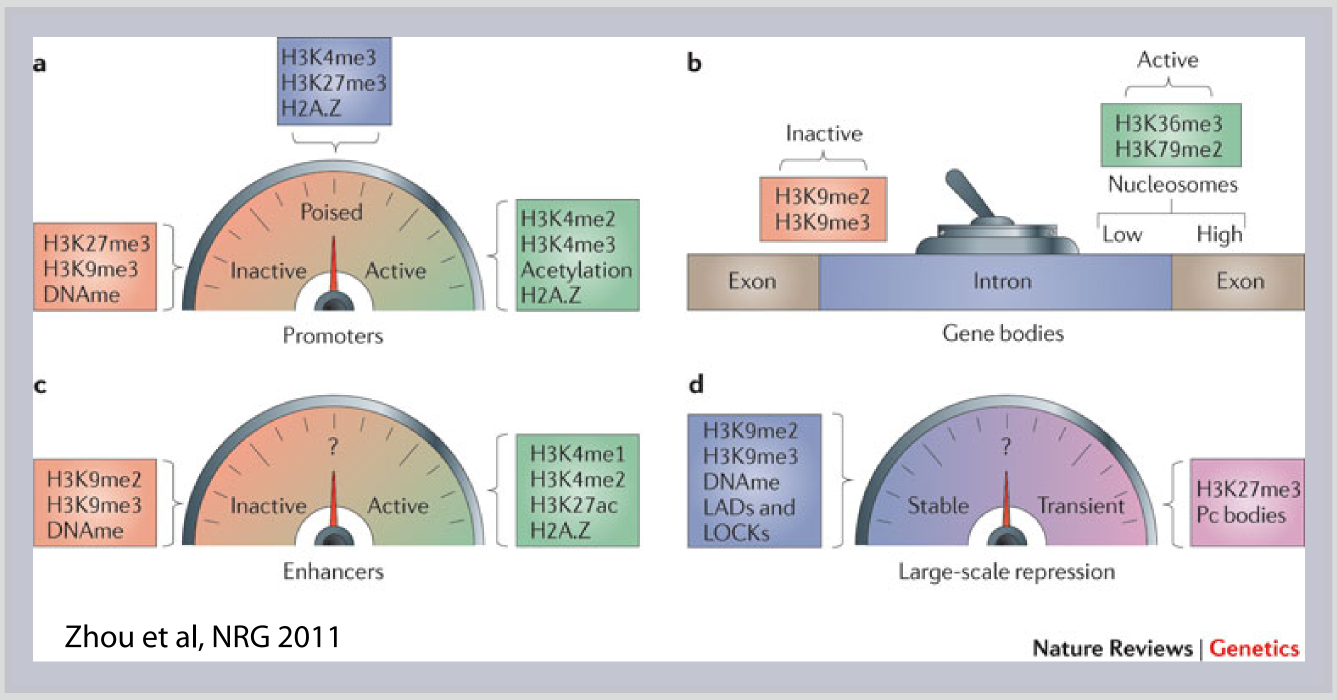
Kosi Gramatikoff, 2007

genomic distribution



Barth and Imhoff, Trends in Bio. Sci 2010

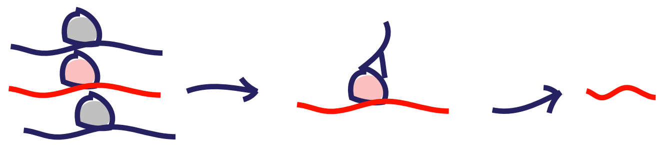
functional association



Zhou et al, NRG 2011

methodology

immunoprecipitation



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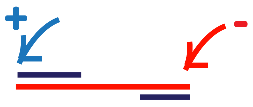
identify tag locations



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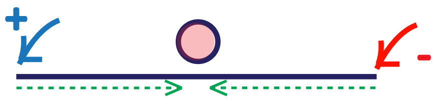

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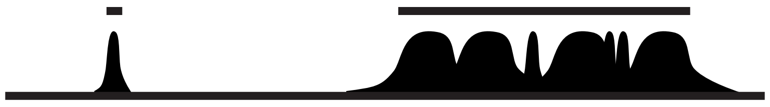

chromatin accessibility

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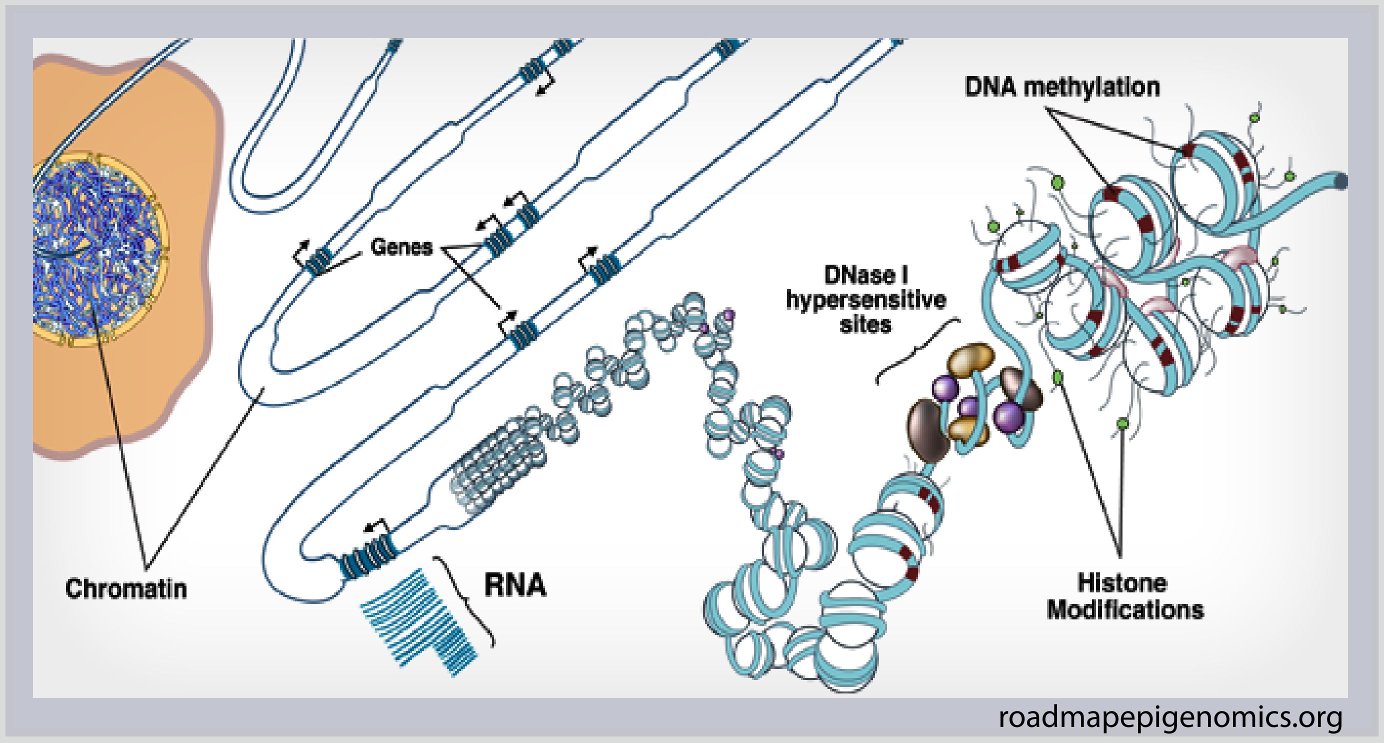
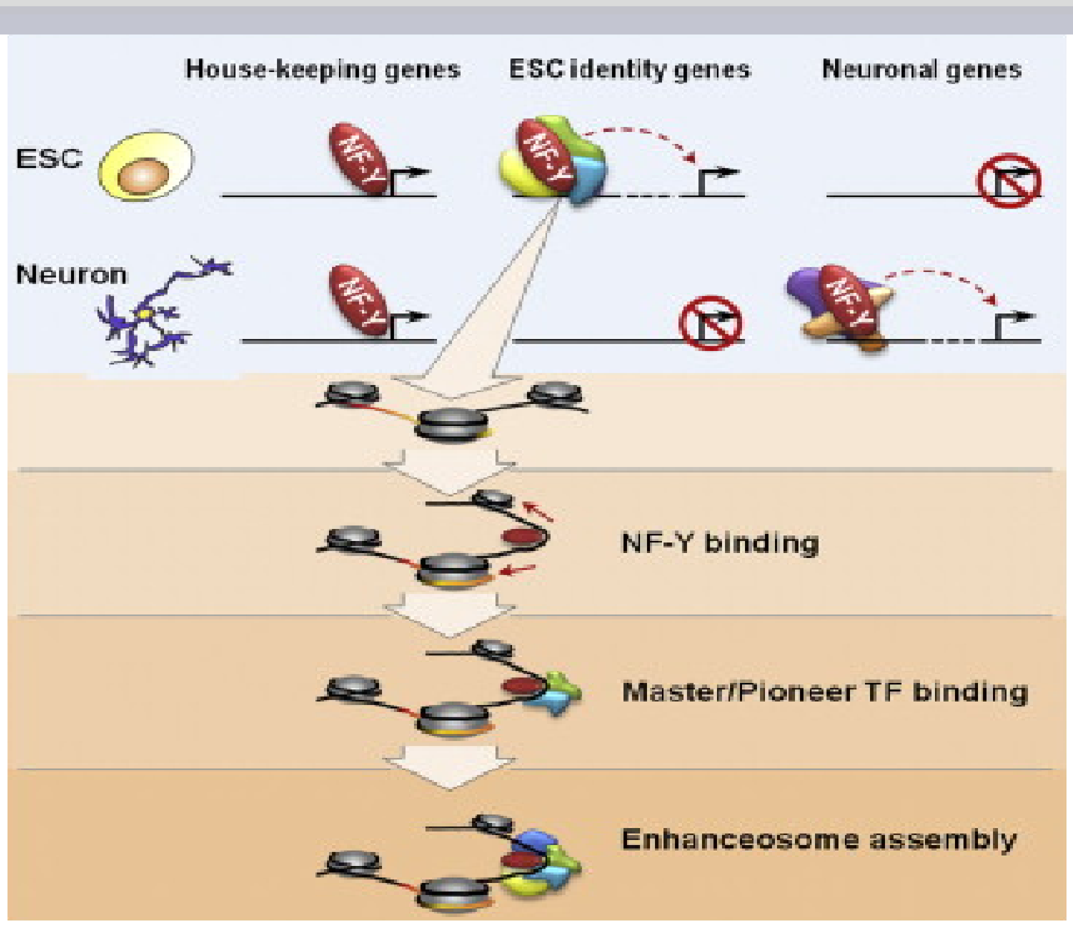
functional regulatory elements are accessible



roadmapepigenomics.org

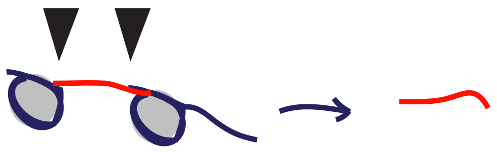
formation of accessible chromatin



Oldfield et al, Mol Cell 2014

methodology (digestion or transposase)

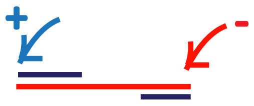
DNase I or Tn5 release fragments of open chromatin



library construction and sequencing

mapping to genome reference (BWA or bowtie2)

identify cleavage/insertion location

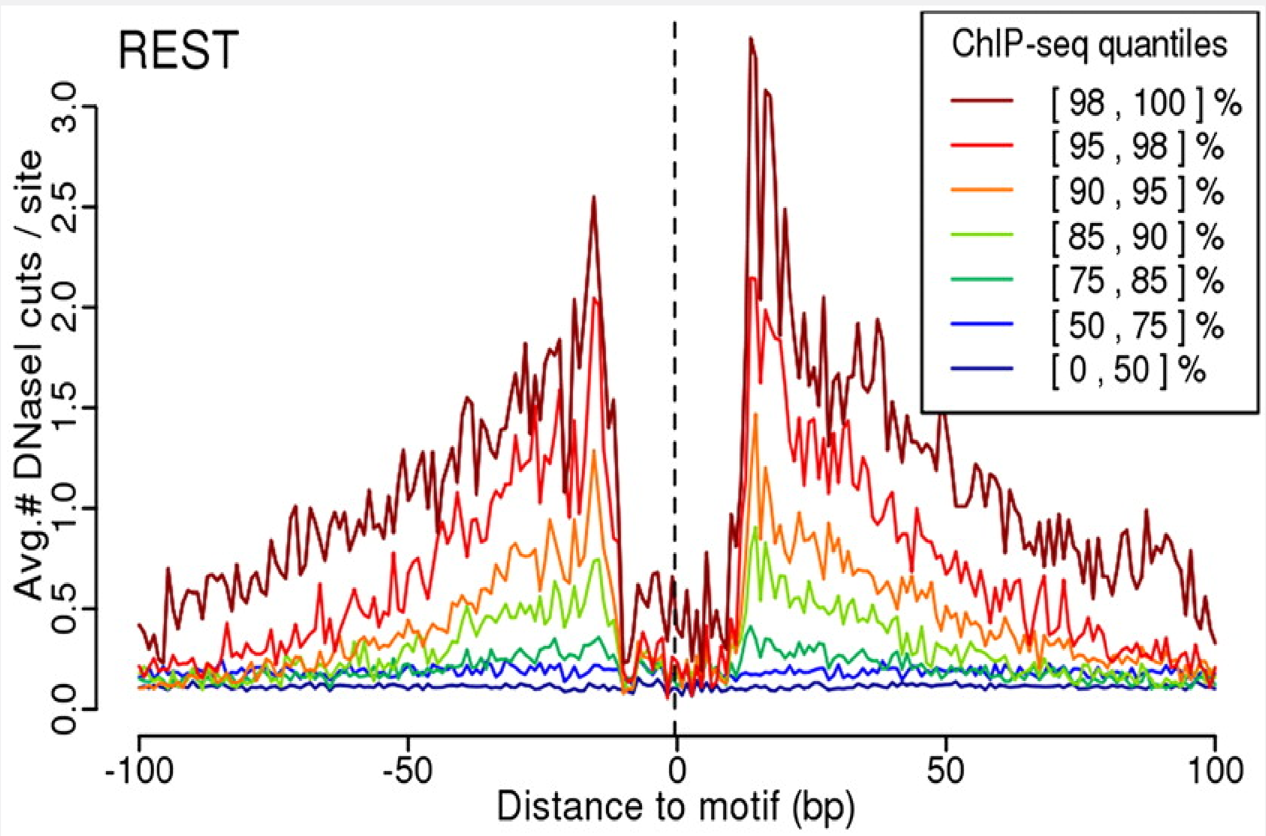


calculate density of cleavage/insertion events

call regions of accessibility

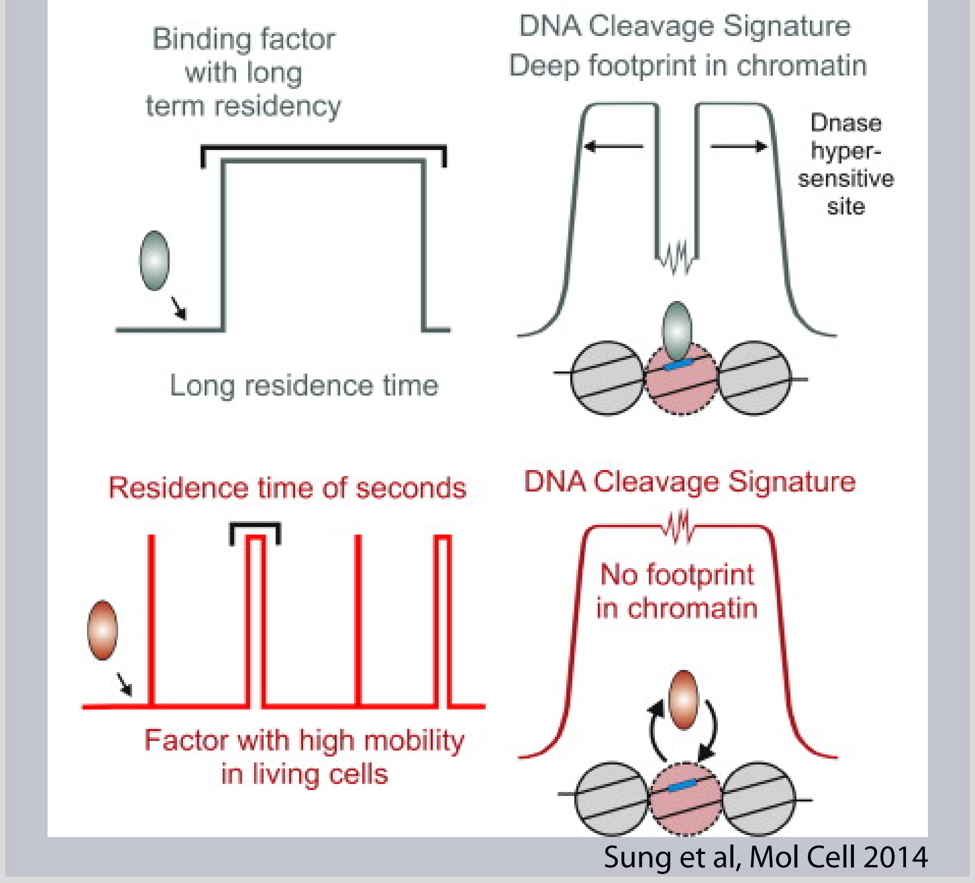
call footprints within accessible chromatin

footprinting



Pique-Regi et al Genome Research 2011

Footprints reflect residence time in chromatin


Binding factor with long term residency
DNA Cleavage Signature Deep footprint in chromatin
Dnase hyper-sensitive site
Long residence time
Residence time of seconds
DNA Cleavage Signature
No footprint in chromatin
Factor with high mobility in living cells

Sung et al, Mol Cell 2014

nucleosome position

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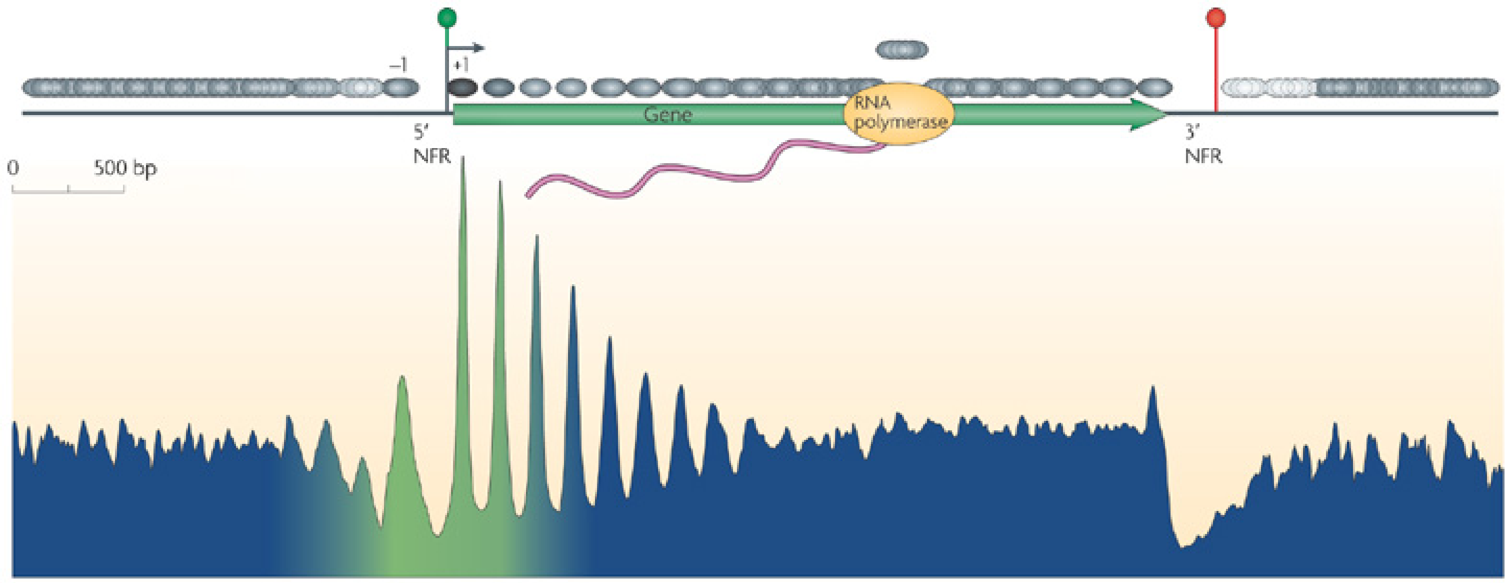
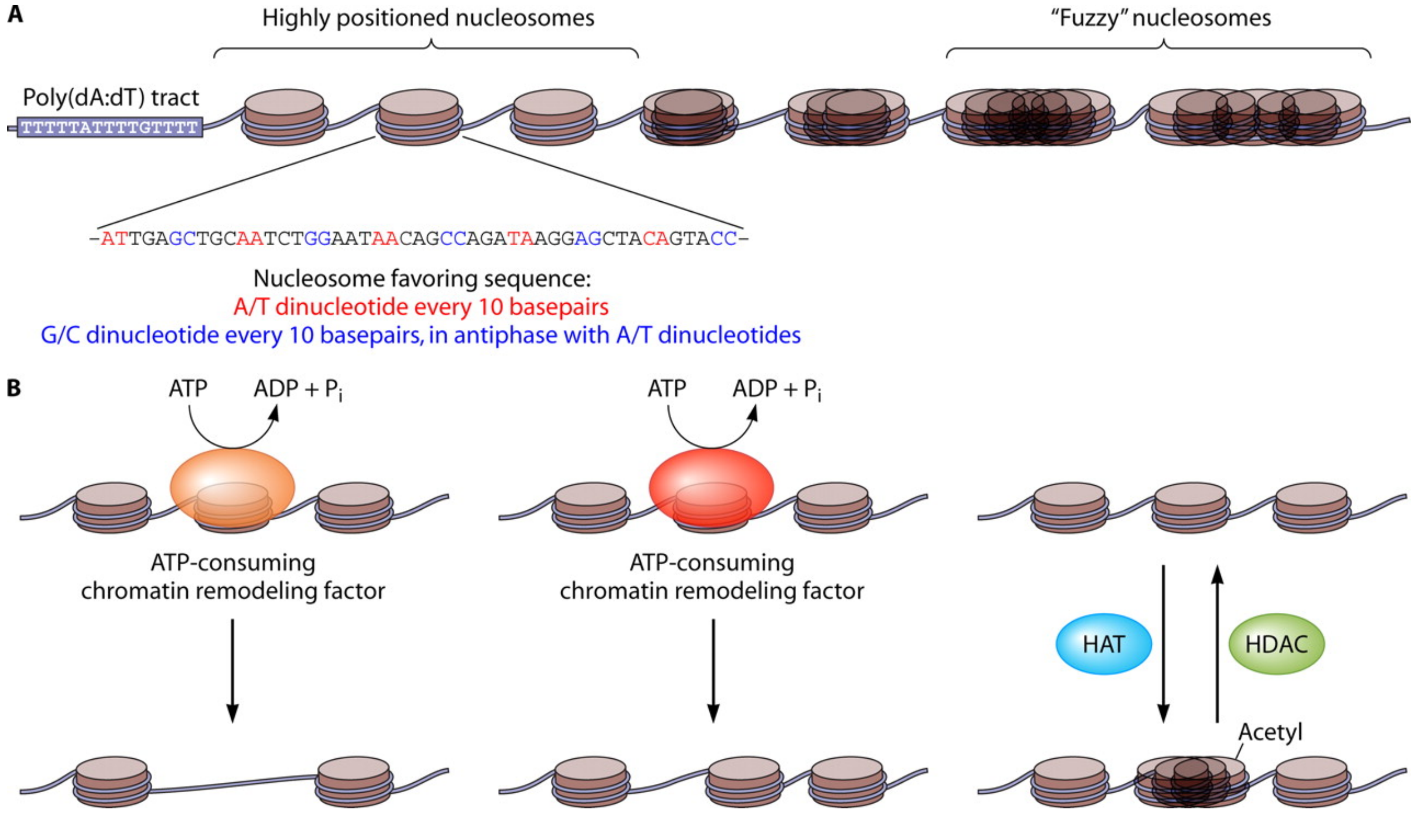
nucleosome positioning during transcription



Nature Reviews | Genetics

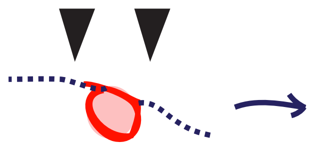
Jiang et al NRG 2009

determinants of positioning stability

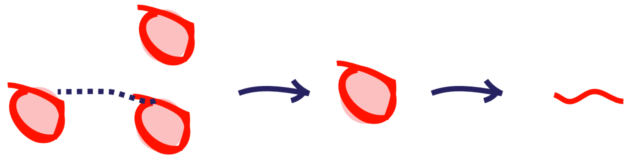

A
Highly positioned nucleosomes
"Fuzzy" nucleosomes
Poly(dA:dT) tract
TTTTTATTTTGTTTT
–ATTGAGCTGCAATCTGGAATAACAGCCAGATAAGGAGCTACAGTACC–
Nucleosome favoring sequence:
A/T dinucleotide every 10 basepairs
G/C dinucleotide every 10 basepairs, in antiphase with A/T dinucleotides
B
ATP
ADP + P_i
ATP-consuming chromatin remodeling factor
ATP
ADP + P_i
ATP-consuming chromatin remodeling factor
HAT
HDAC
Acetyl

methodology (MNase digestion)

MNase I digests linker DNA, releasing multisomes



purify mononucleosome-bound DNA



library construction and sequencing

mapping to genome reference (BWA or bowtie2)

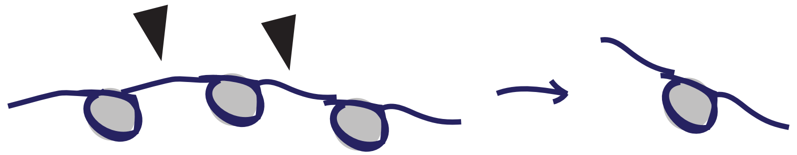
identify cleavage location

calculate nucleosomal density

call positioned nucleosomes

methodology (transposon insertion)

Tn5 integrates into accessible chromatin, and releases multisomes



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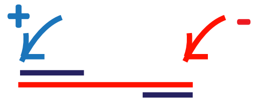
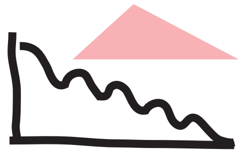


identify reads that span at least one nucleosome



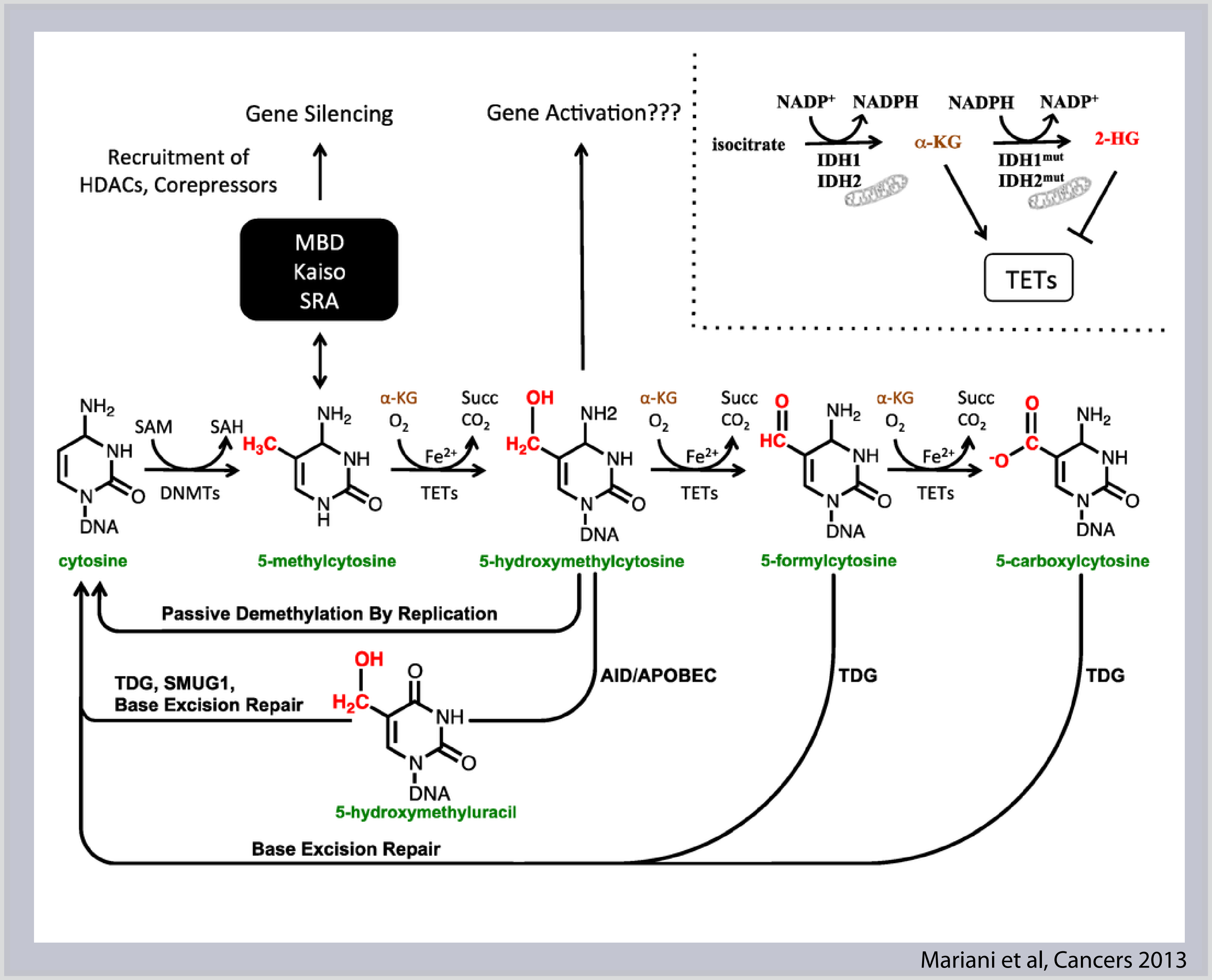
read length

calculate density of tags / number of insertion events

call positioned nucleosomes

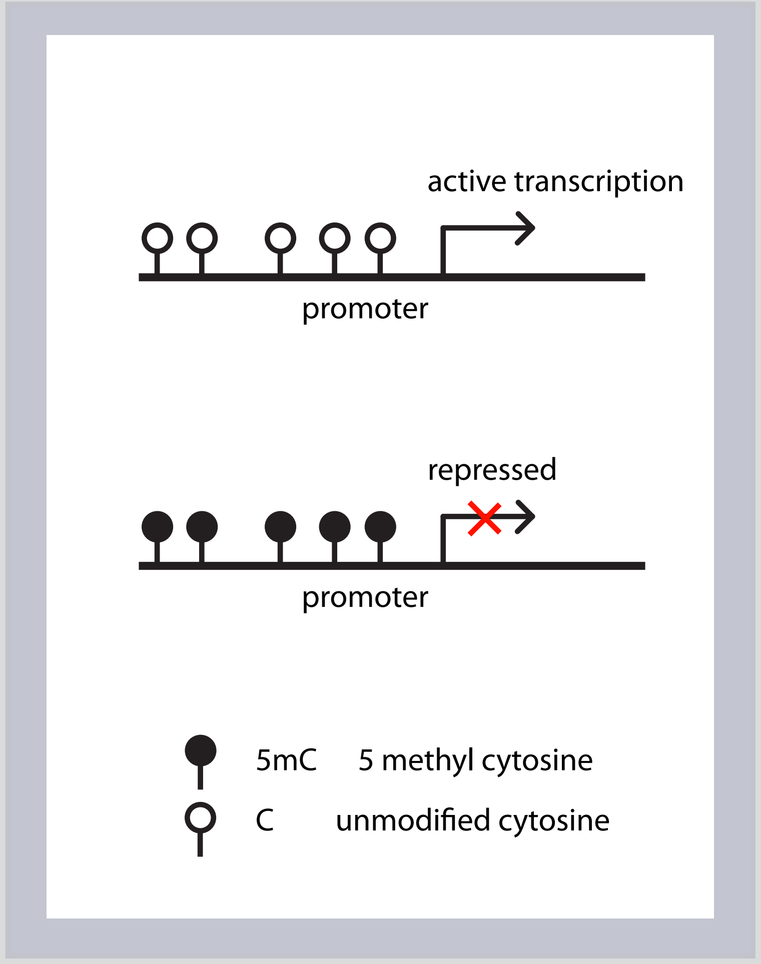
DNA modifications

catalogue of common base modifications (in mammals)

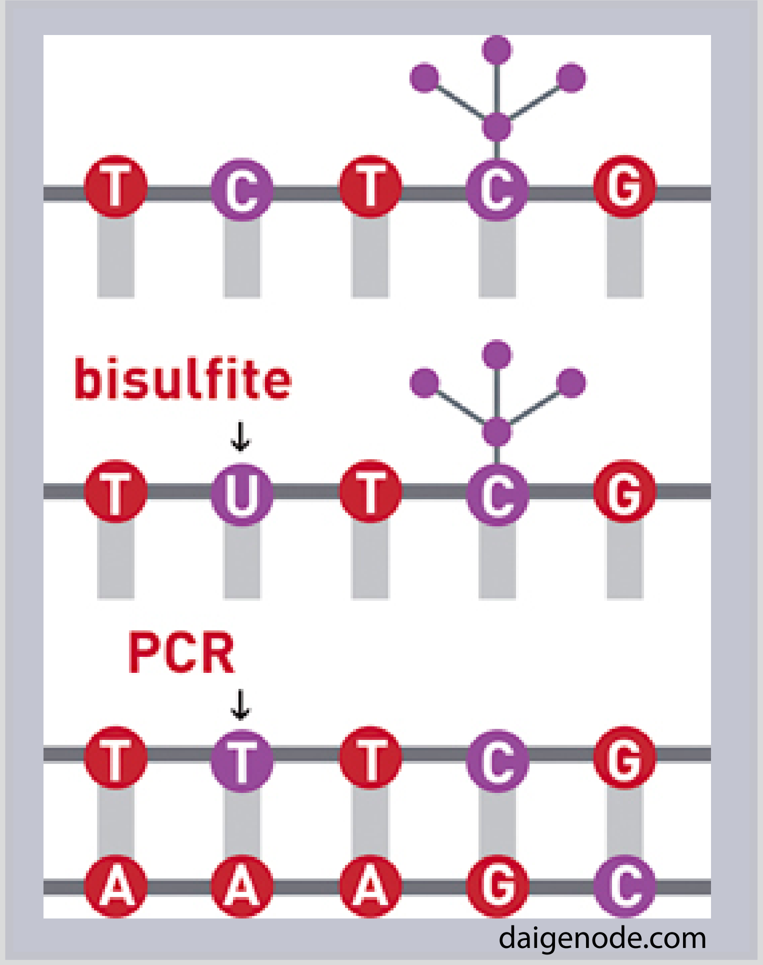


Mariani et al, Cancers 2013

5mC repression of gene expression



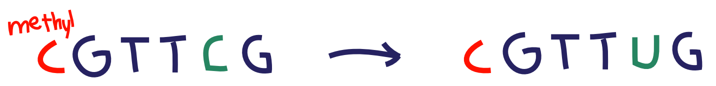
bisulfite conversion / protection



daigenode.com

methodology

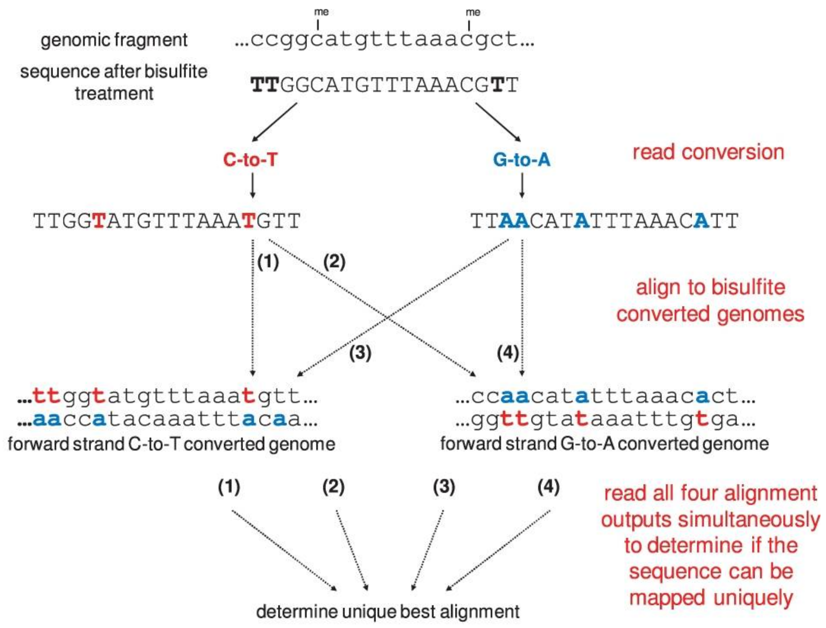
convert DNA with bisulfite



fragmentation

library construction and sequencing

mapping


genomic fragment
me
me
...ccggcatgtttaaacgct...
sequence after bisulfite treatment
TTGGCATGTTTAAACGTT
C-to-T
G-to-A
read conversion
TTGGTATGTTTAAATGTT
TTAACATATTTAAACATT
(1)
(2)
(3)
(4)
align to bisulfite converted genomes
...ttggtatgtttaaatgtt...
...aaccatacaaatttacaa...
forward strand C-to-T converted genome
...ccaacatatttaaacact...
...ggttgtataaatttgtga...
forward strand G-to-A converted genome
(1)
(2)
(3)
(4)
read all four alignment outputs simultaneously to determine if the sequence can be mapped uniquely
determine unique best alignment

Krueger et al Bioinformatics 2011

calculate % of tags methylated per genomic position

DNA-associated factors

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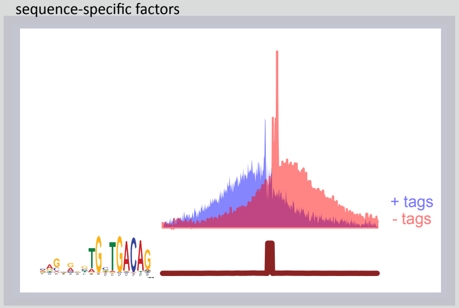
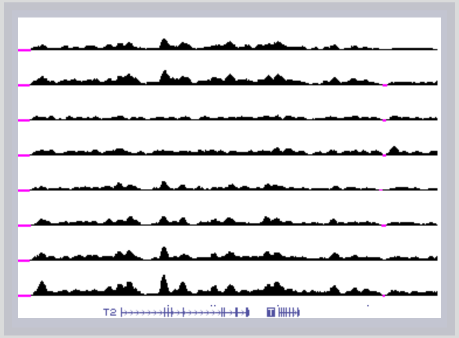
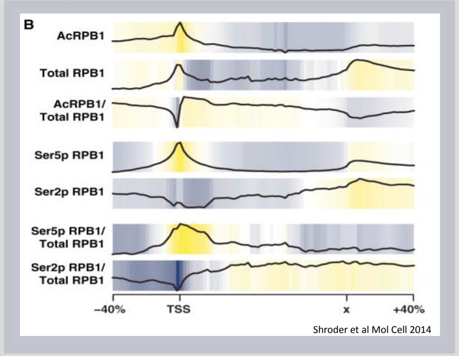
sequence-specific factors



chromatin associated



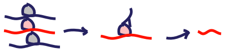
transcription machinery



Shroder et al Mol Cell 2014

methodology

immunoprecipitation

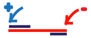


library construction

sequencing

mapping to genome reference (BWA or bowtie2)

identify tag locations



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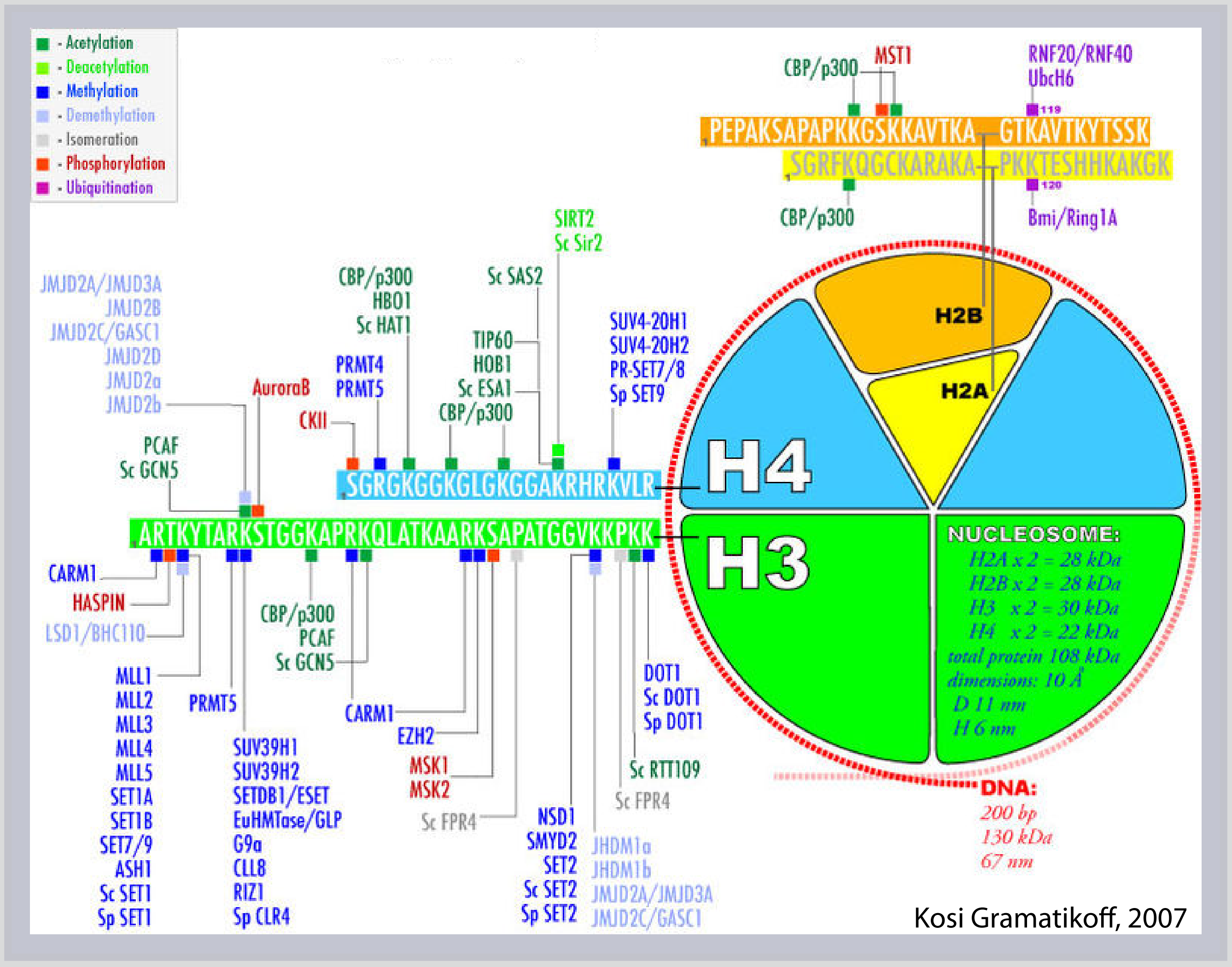
histone modifications

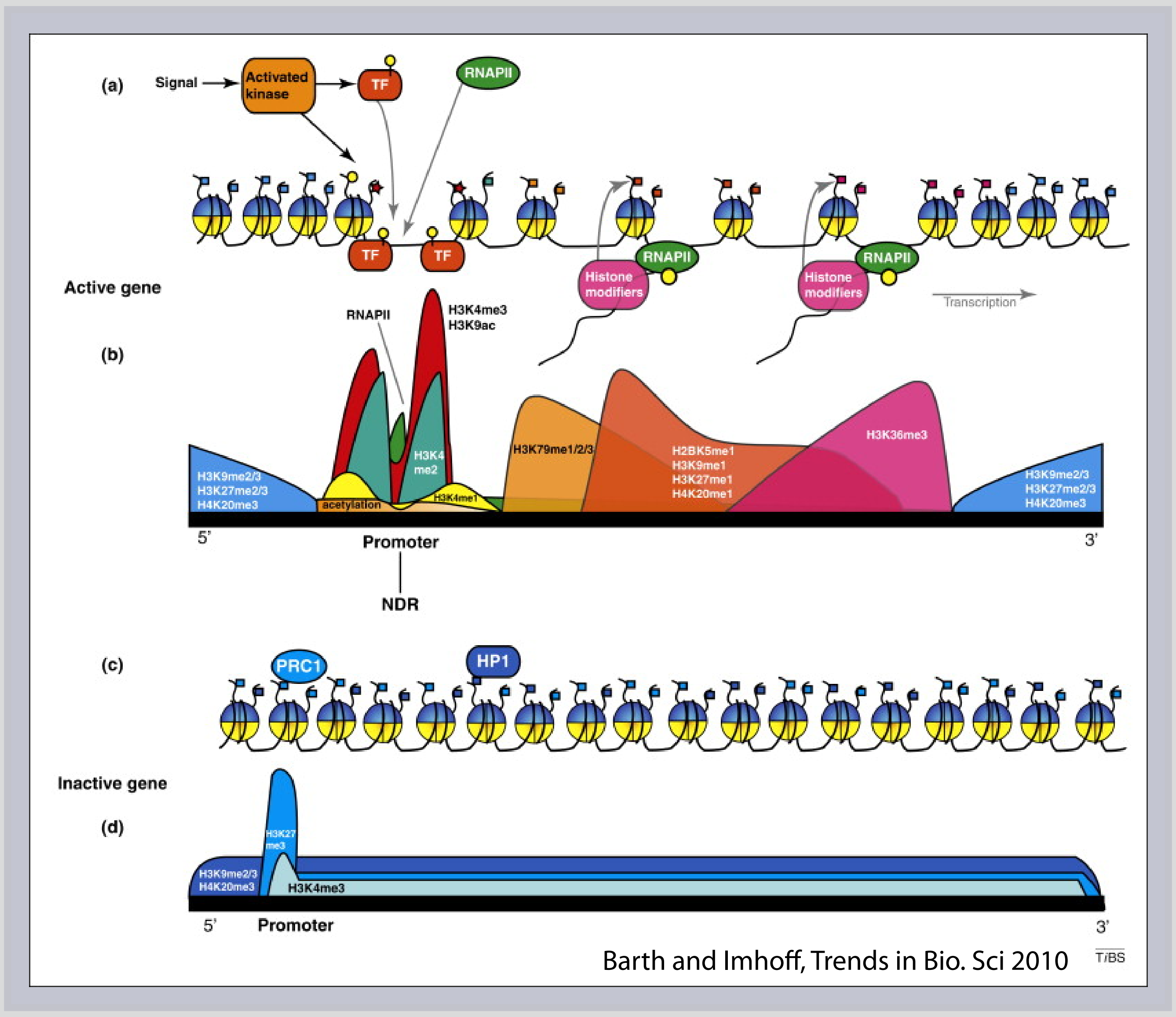
BMI206 November 3, 2014

catalogue



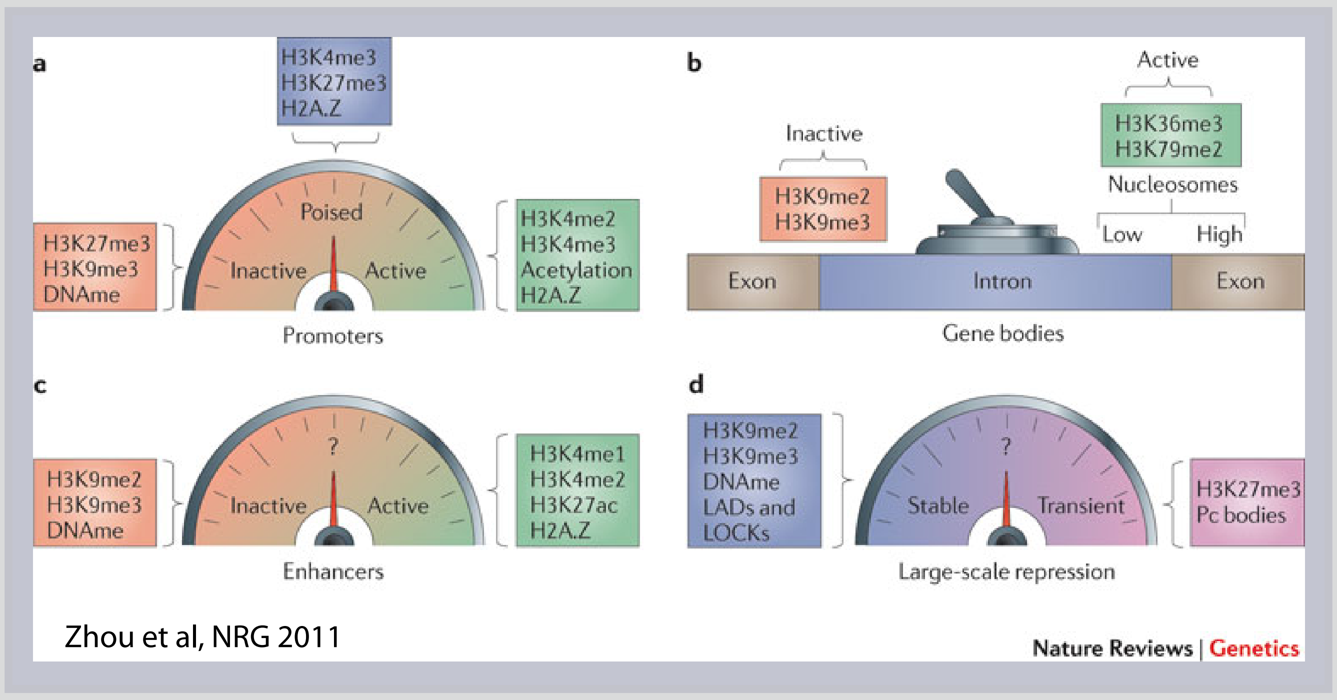
Kosi Gramatikoff, 2007

genomic distribution



Barth and Imhoff, Trends in Bio. Sci 2010

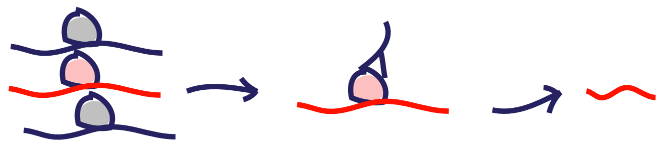
functional association



Zhou et al, NRG 2011

methodology

immunoprecipitation



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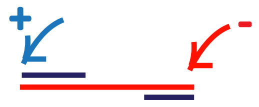

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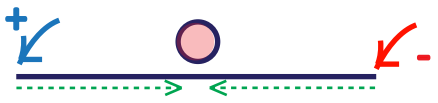

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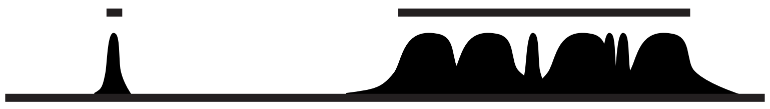
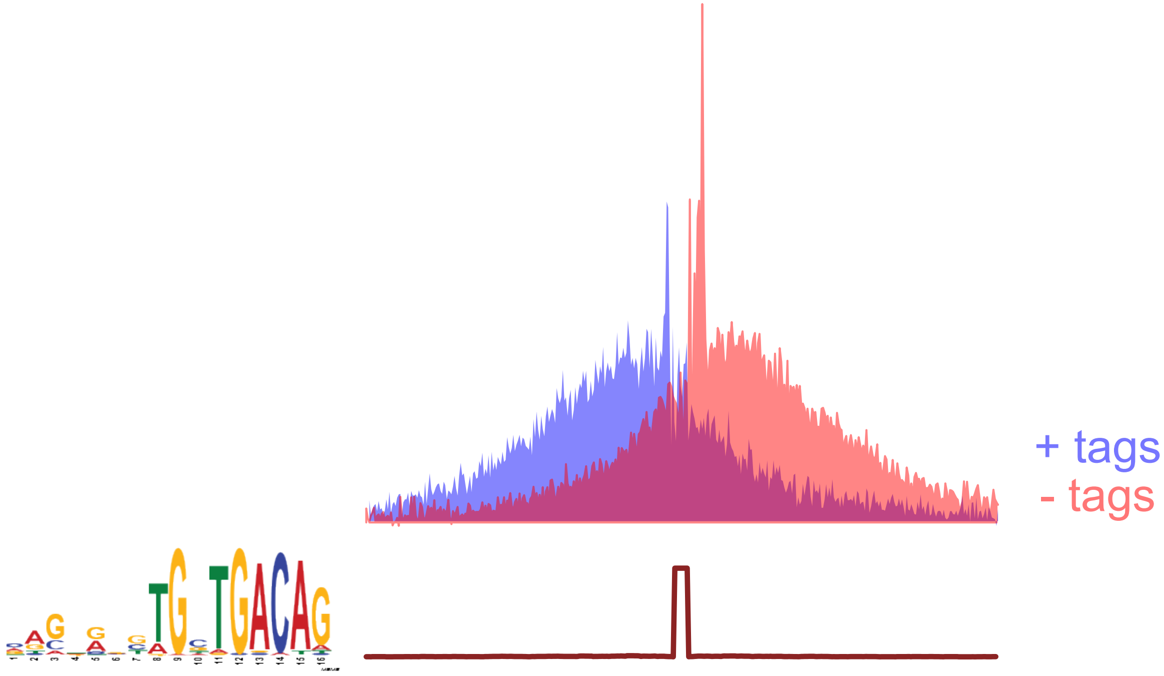


sequence-specific factors



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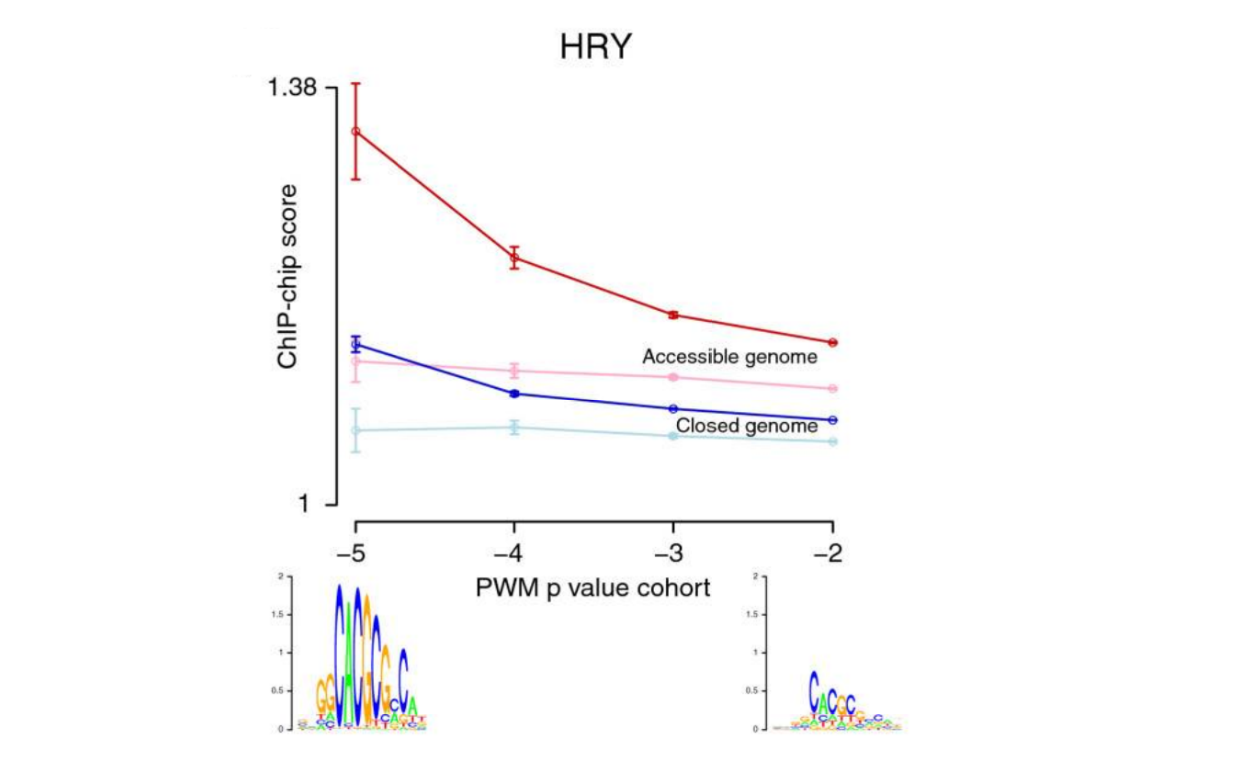
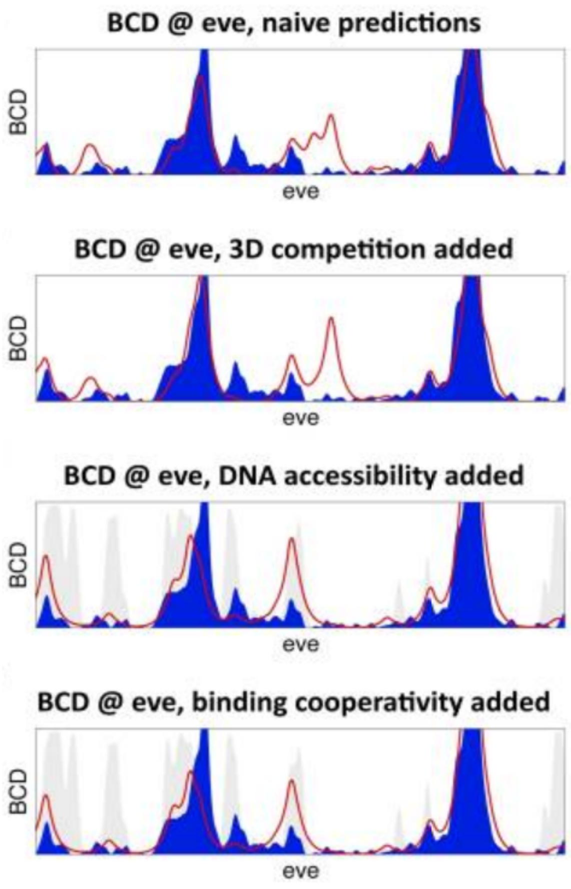


Li et al Genome Biology, 2011

sequence-specific factors



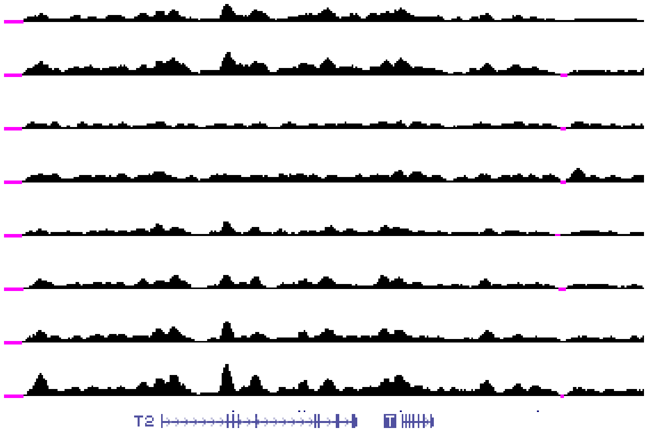
━ modeled ChIP tag density

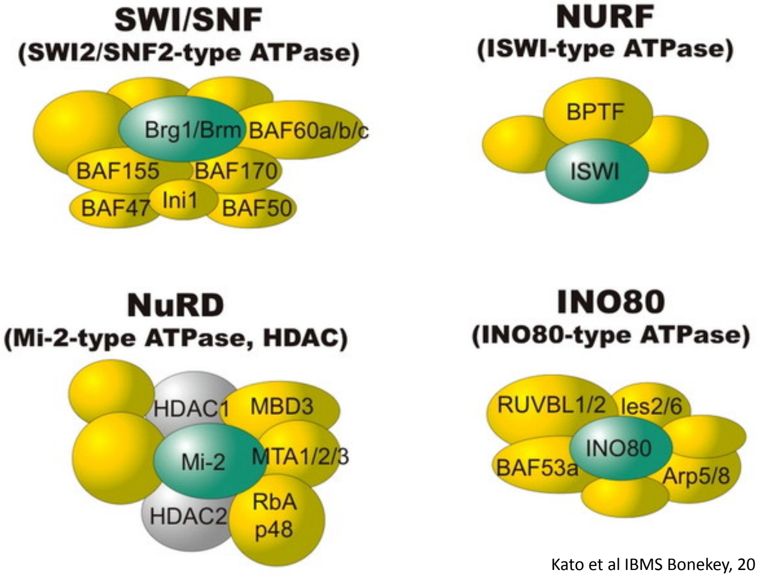
■ actual ChIP tag density

Kaplan et al PLoS Genetics, 2011

chromatin-associated factors

chromatin remodellers



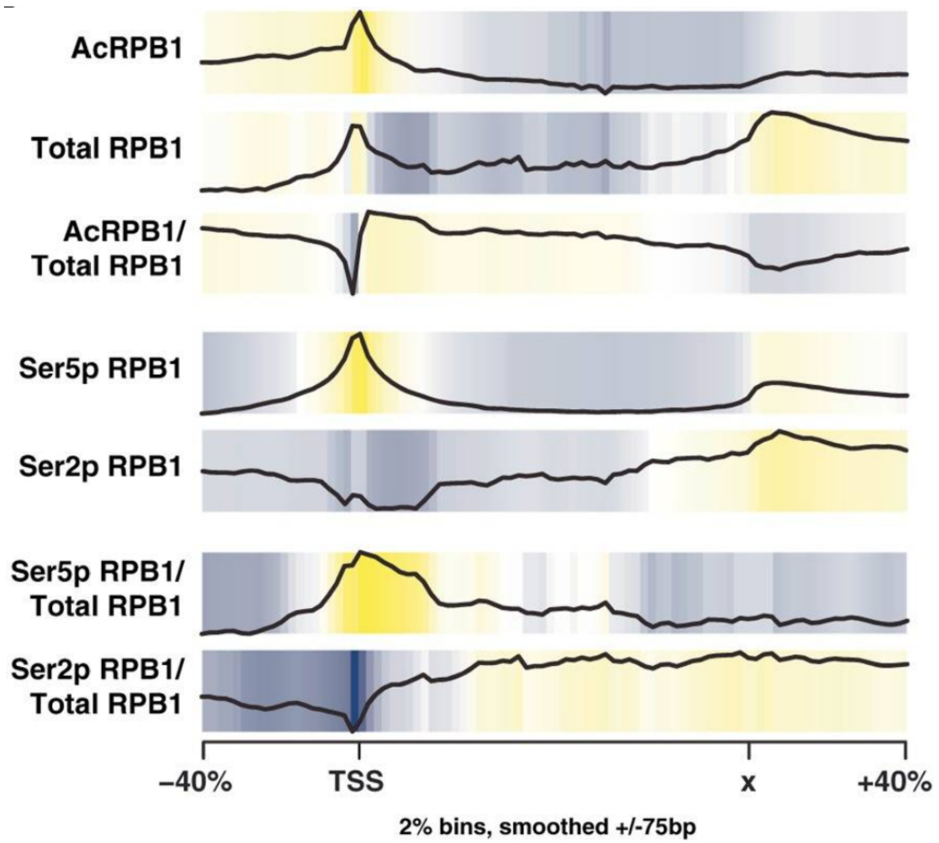


Kato et al IBMS Bonekey, 2010

transcription machinery

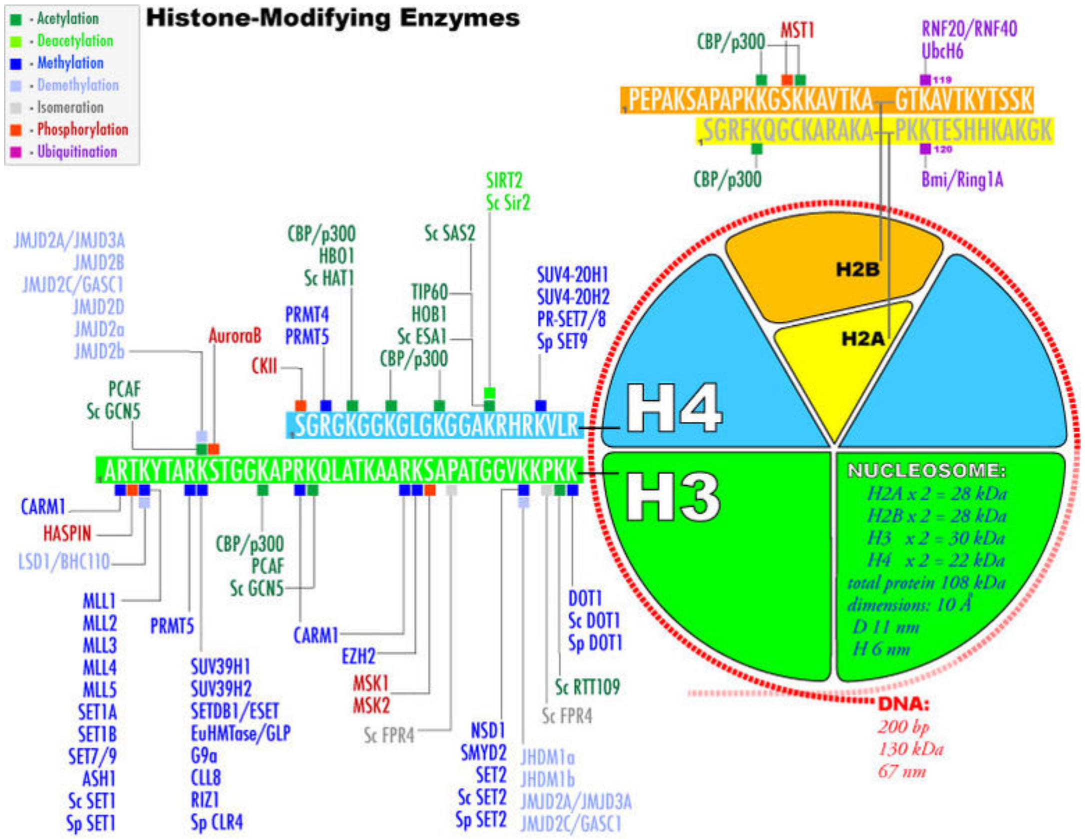
e.g. RNA polymerase II

(and various post-translational modifications)



Schroder et al Mol Cell 2012

histone modifications



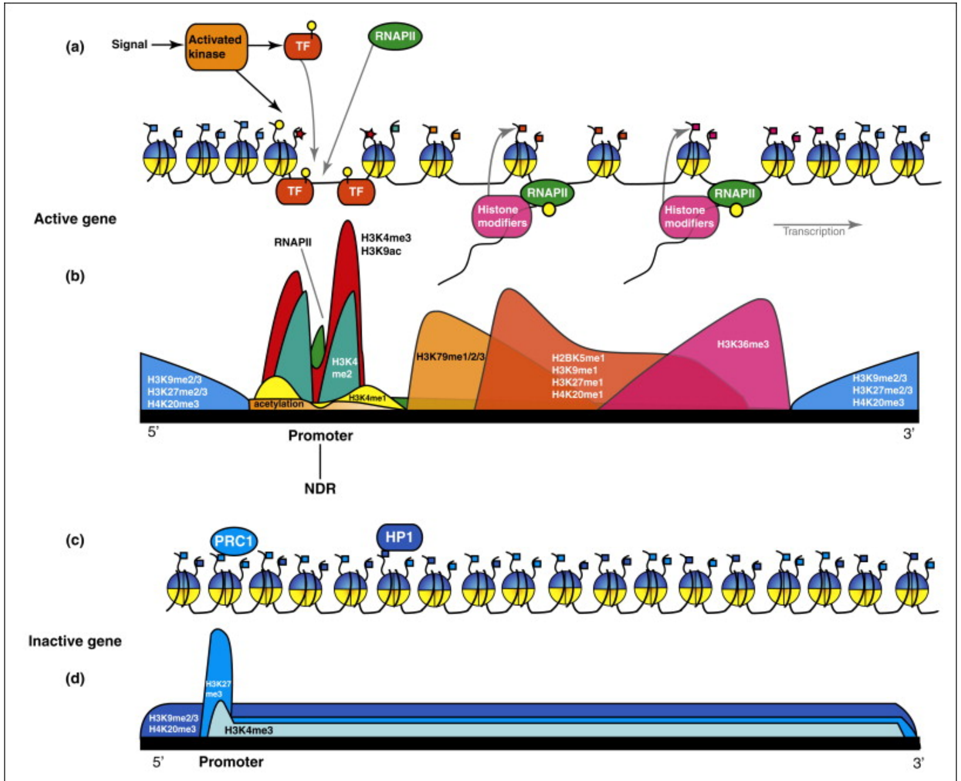
Kosi Gramatikoff, 2007

most commonly studied:

H3K4me3
H3K27ac
H3K27me3

histone modifications

wide range of binding patterns

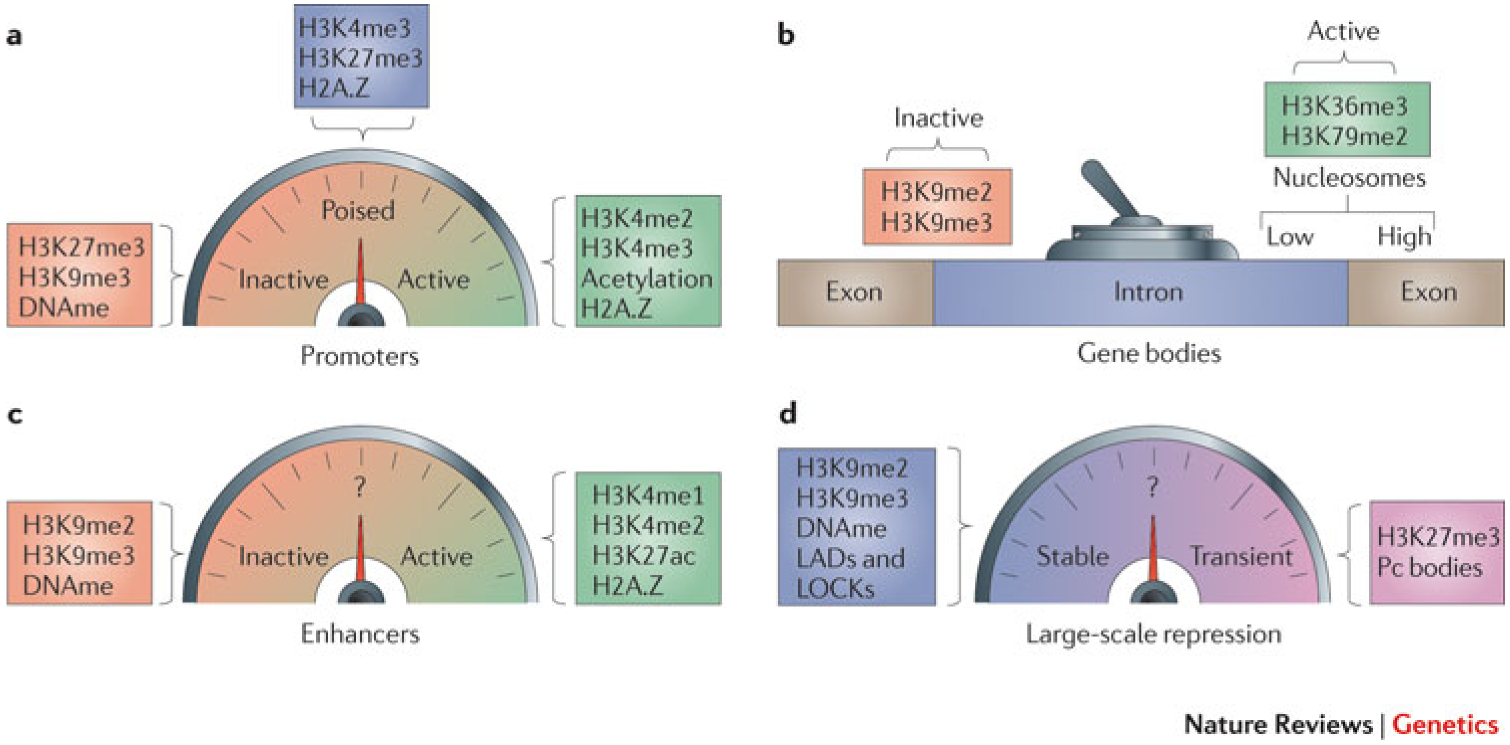


Barth and Imhoff, Trends in Bio. Sci 2010

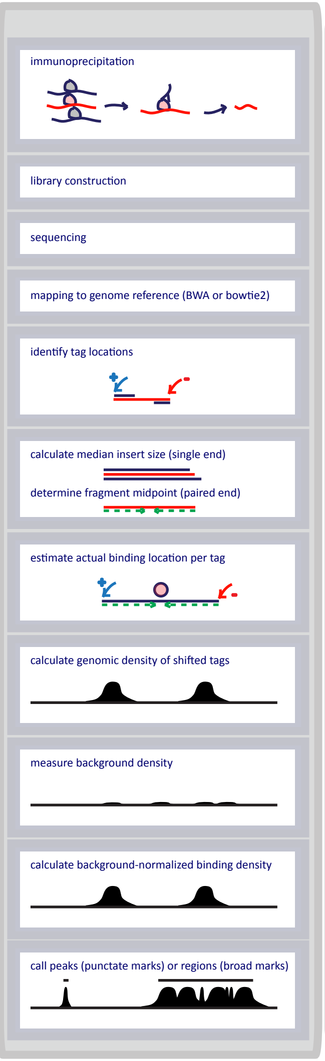
H3K4me3 - transcription start sites
H3K27ac - active enhancers and TSS
H3K27me3 - domain around TSS
H3K36me3 - active gene bodies

histone modifications

wide range of functional associations



Zhou et al, NRG 2011



immunoprecipitation

library construction

sequencing

mapping to genome reference (BWA or bowtie2)

identify tag locations

calculate median insert size (single end)

determine fragment midpoint (paired end)

estimate actual binding location per tag

calculate genomic density of shifted tags

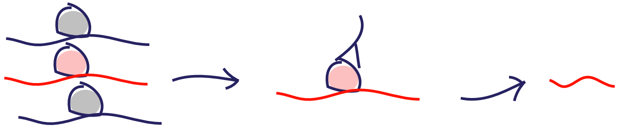
measure background density

calculate background-normalized binding density

call peaks (punctate marks) or regions (broad marks)

methodology

immunoprecipitation



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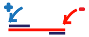

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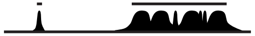
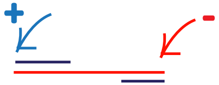


methodology

identify tag locations



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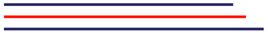
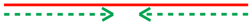
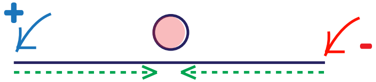


determine fragment midpoint (paired end)



estimate actual binding location per tag



immunoprecipitation

library construction

sequencing

mapping to genome reference (BWA or bowtie2)

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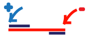

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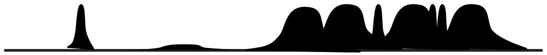
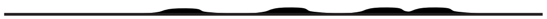
methodology

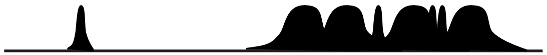
calculate genomic density of shifted tags



measure background density



calculate background-normalized binding density



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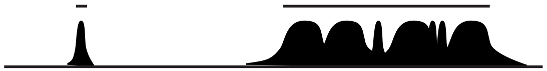
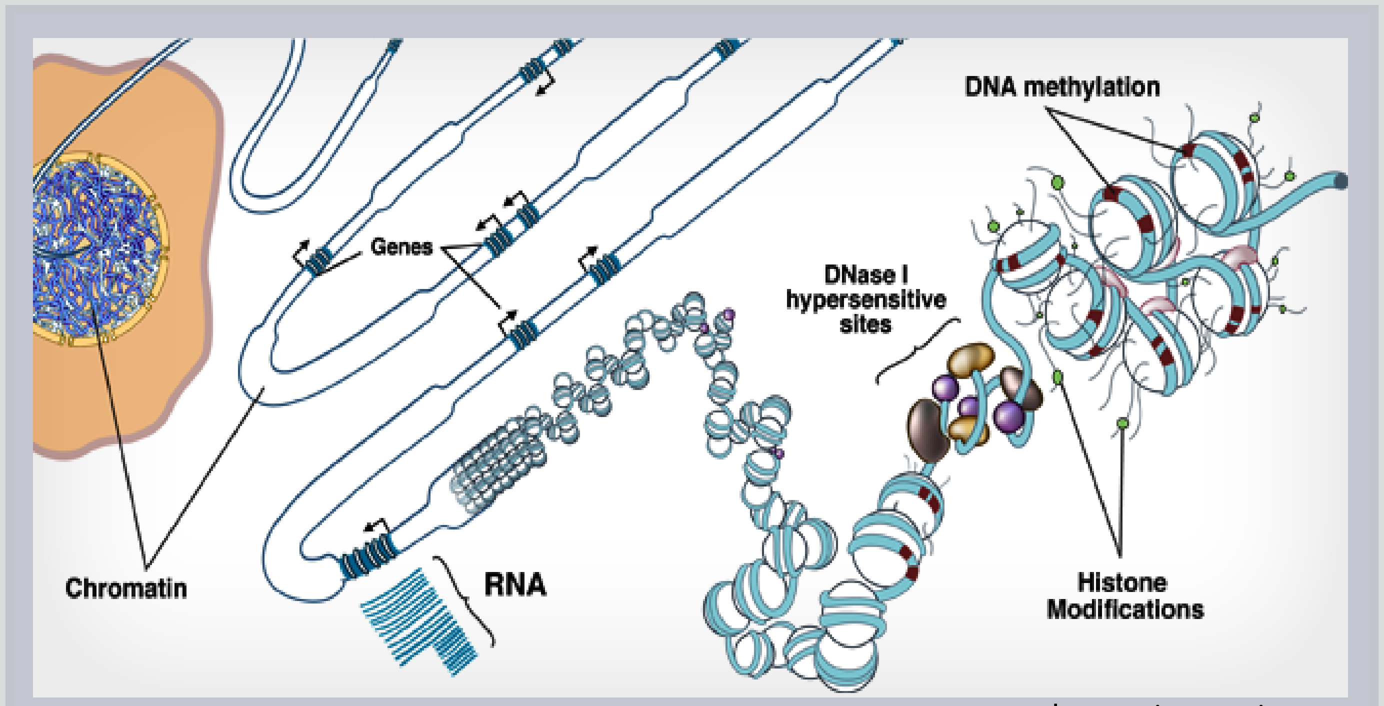


chromatin accessibility

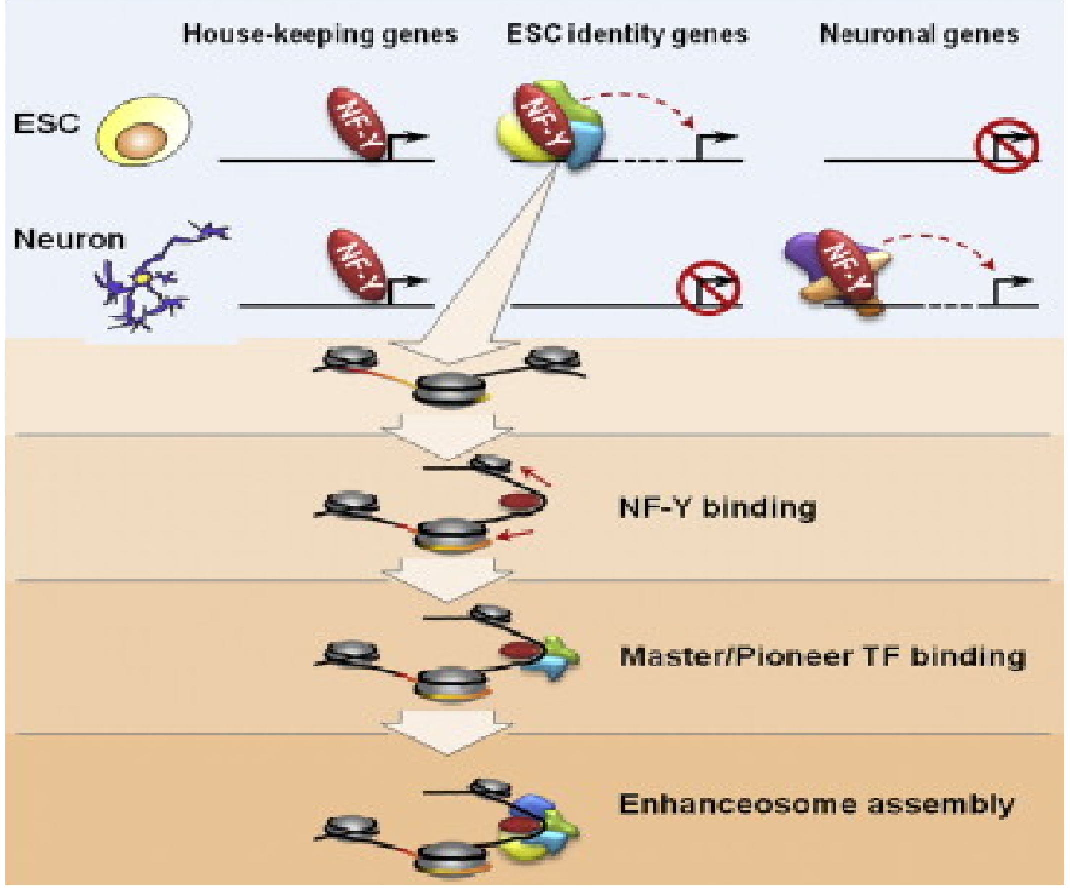
BMI206 November 3, 2014

functional regulatory elements are accessible



roadmapepigenomics.org

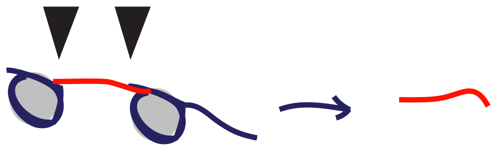
formation of accessible chromatin



Oldfield et al, Mol Cell 2014

methodology (digestion or transposase)

DNase I or Tn5 release fragments of open chromatin



library construction and sequencing

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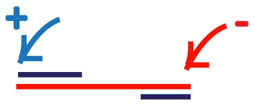
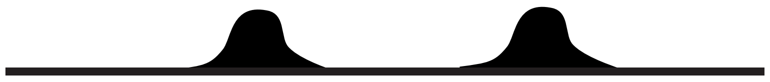
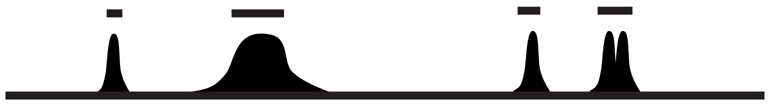


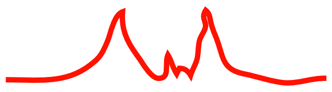
calculate density of cleavage/insertion events



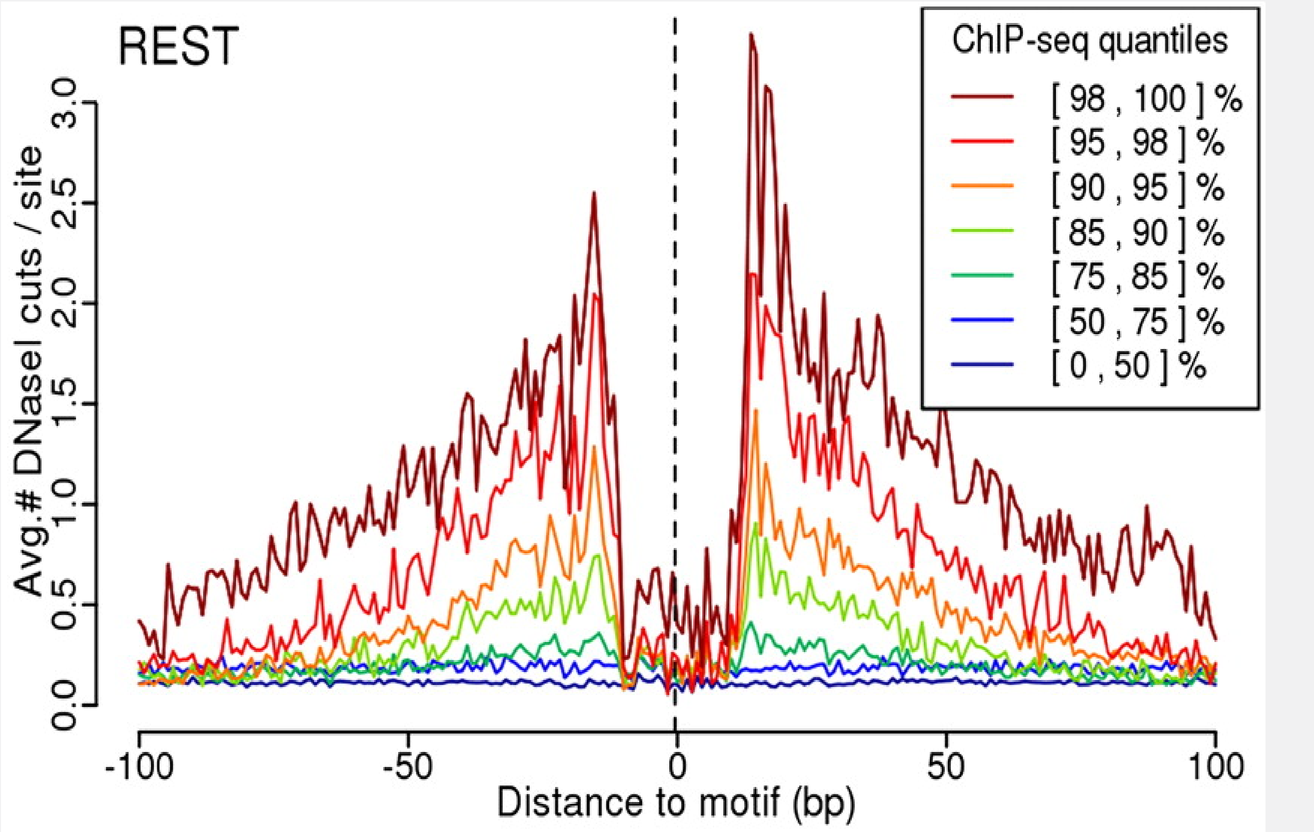
call regions of accessibility



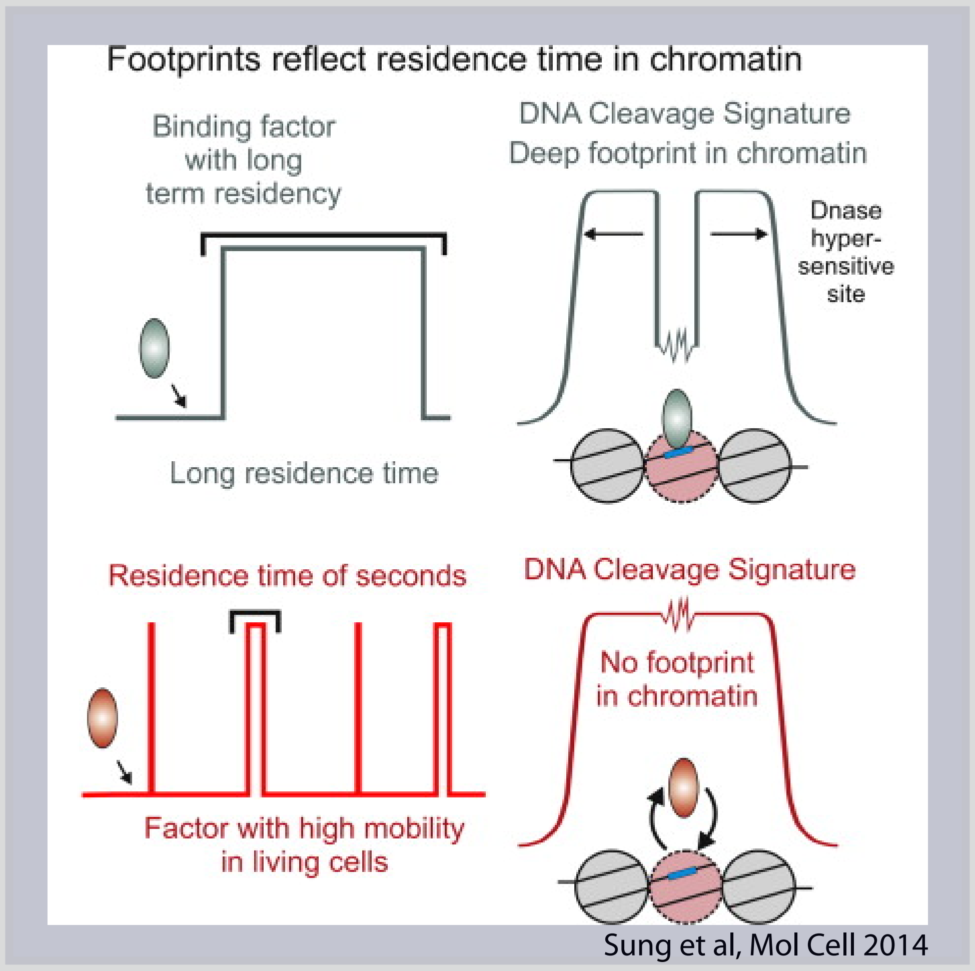
call footprints within accessible chromatin



footprinting

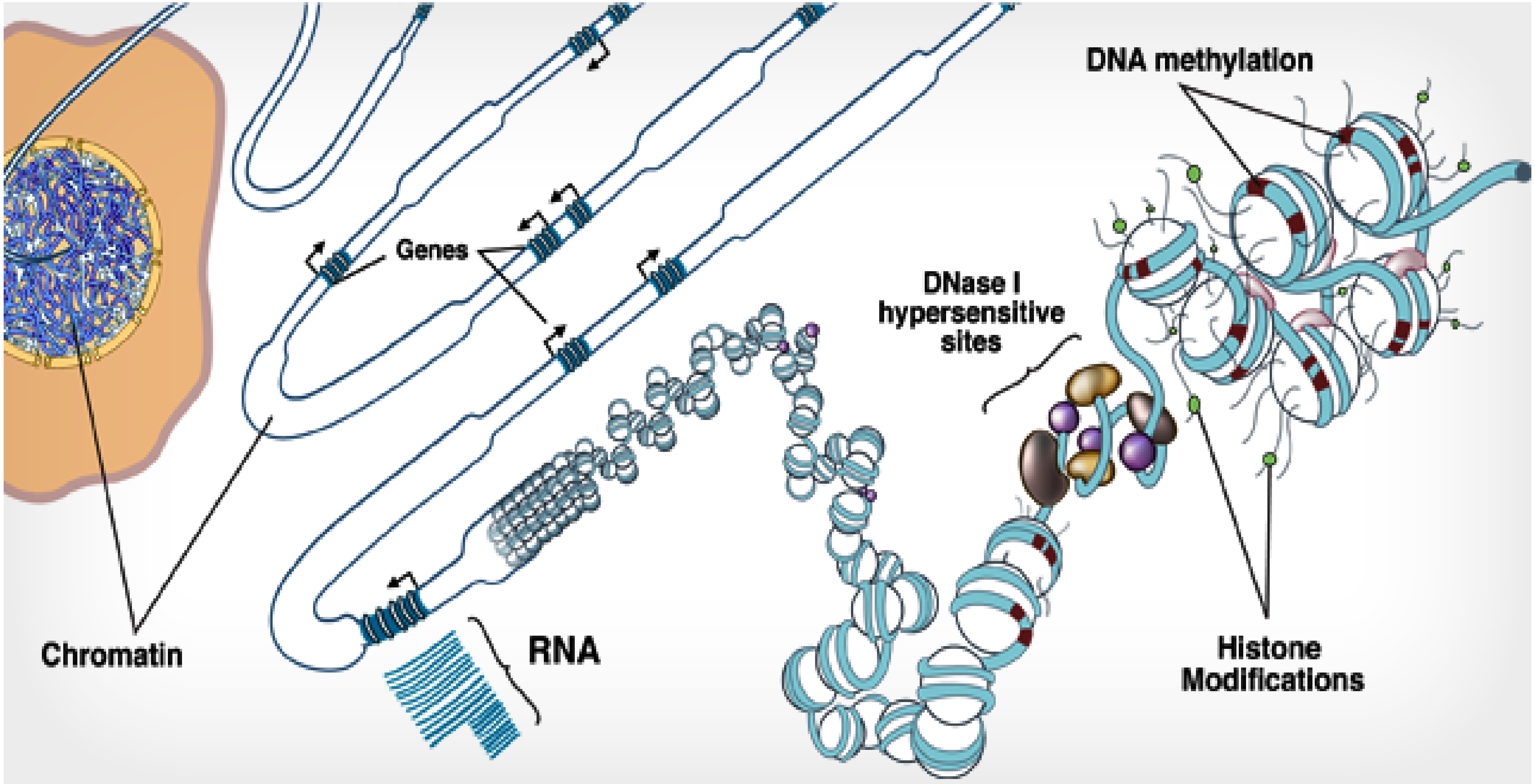


Pique-Regi et al Genome Research 2011


Footprints reflect residence time in chromatin
Binding factor with long term residency
DNA Cleavage Signature
Deep footprint in chromatin
Dnase hyper-sensitive site
Long residence time
Residence time of seconds
DNA Cleavage Signature
No footprint in chromatin
Factor with high mobility in living cells

Sung et al, Mol Cell 2014

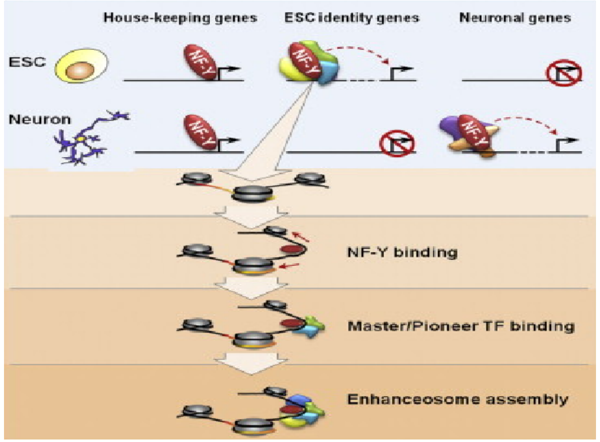
functional regulatory elements are accessible



roadmapepigenomics.org

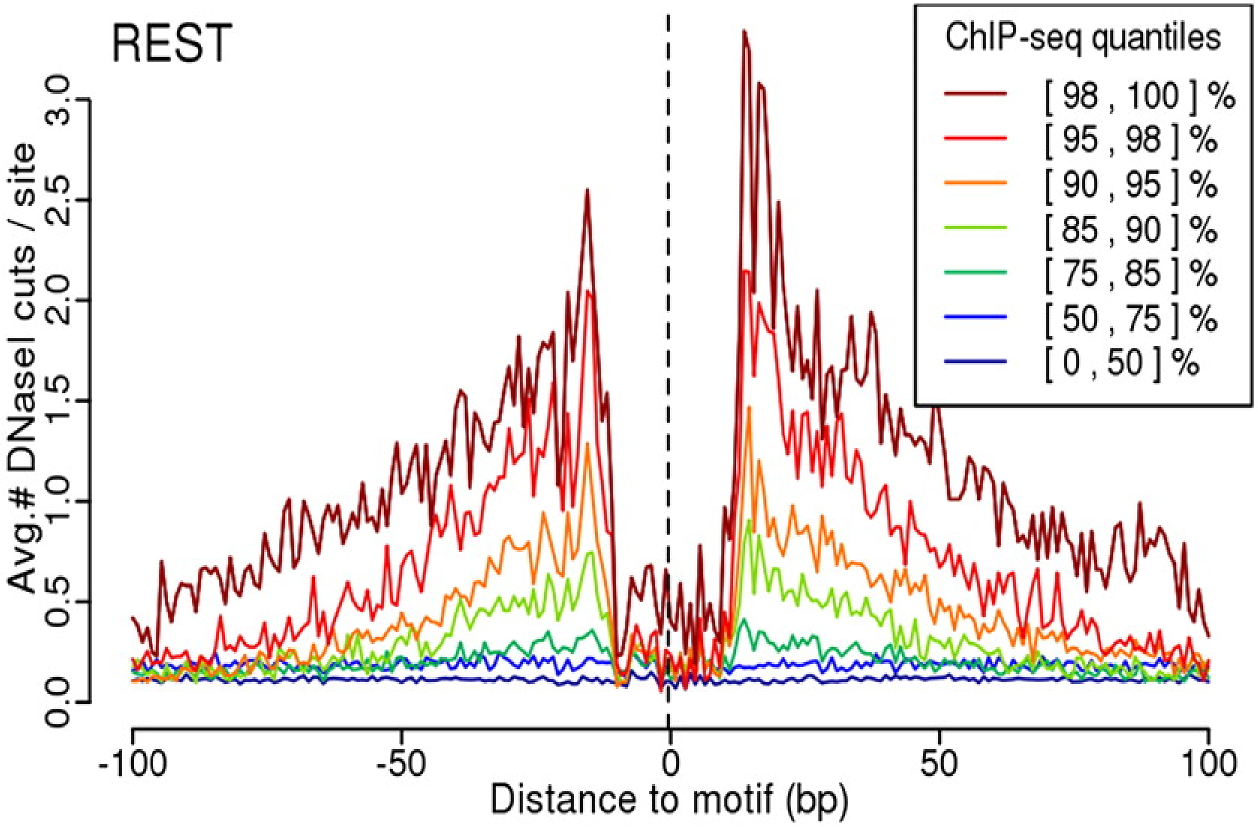
formation of accessible chromatin

pioneering factors make important genes accessible during development



Oldfield et al, Mol Cell 2014

footprinting

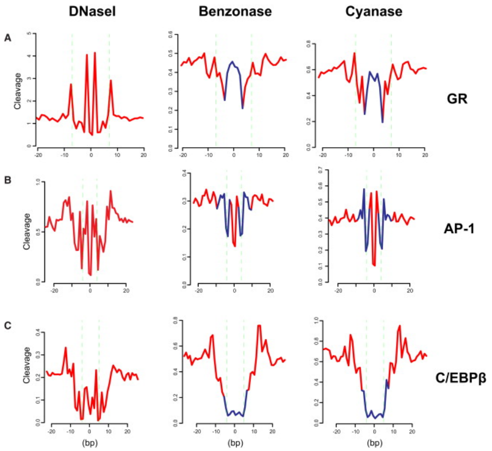


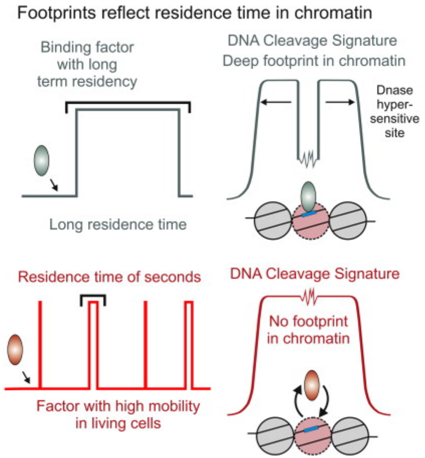
Pique-Regi et al Genome Research 2011

footprinting

distinct for
different factors

not consistently
present for all factors

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Residence time of seconds
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No footprint in chromatin
Factor with high mobility in living cells

(effect seen in naked DNA for many factors)

Sung et al, Mol Cell 2014

methodology (digestion or transposase)

library construction and sequencing

mapping to genome reference (BWA or bowtie2)

identify cleavage/insertion location

calculate density of cleavage/insertion events

call regions of accessibility

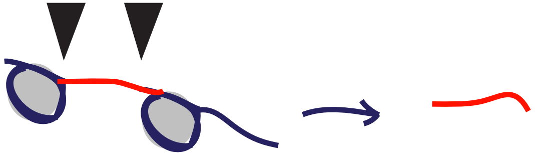
call footprints within accessible chromatin

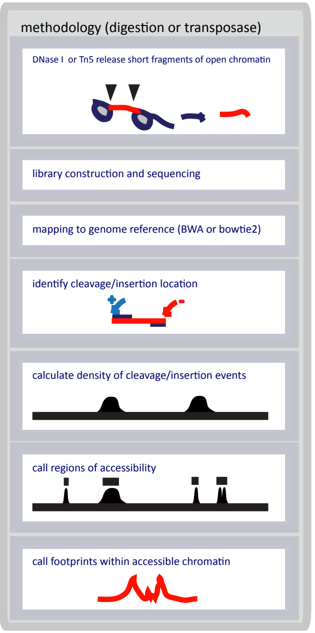
methodology

DNase I digestion
(DNase-seq)

transposon integration
(ATAC-seq)

DNase I or Tn5 release short fragments of open chromatin





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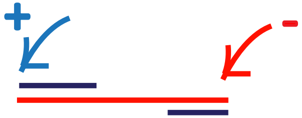

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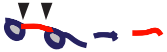

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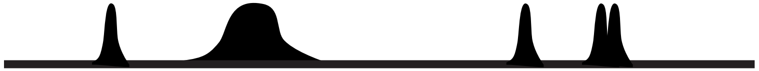

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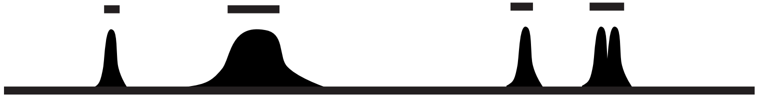

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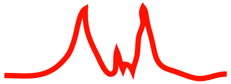
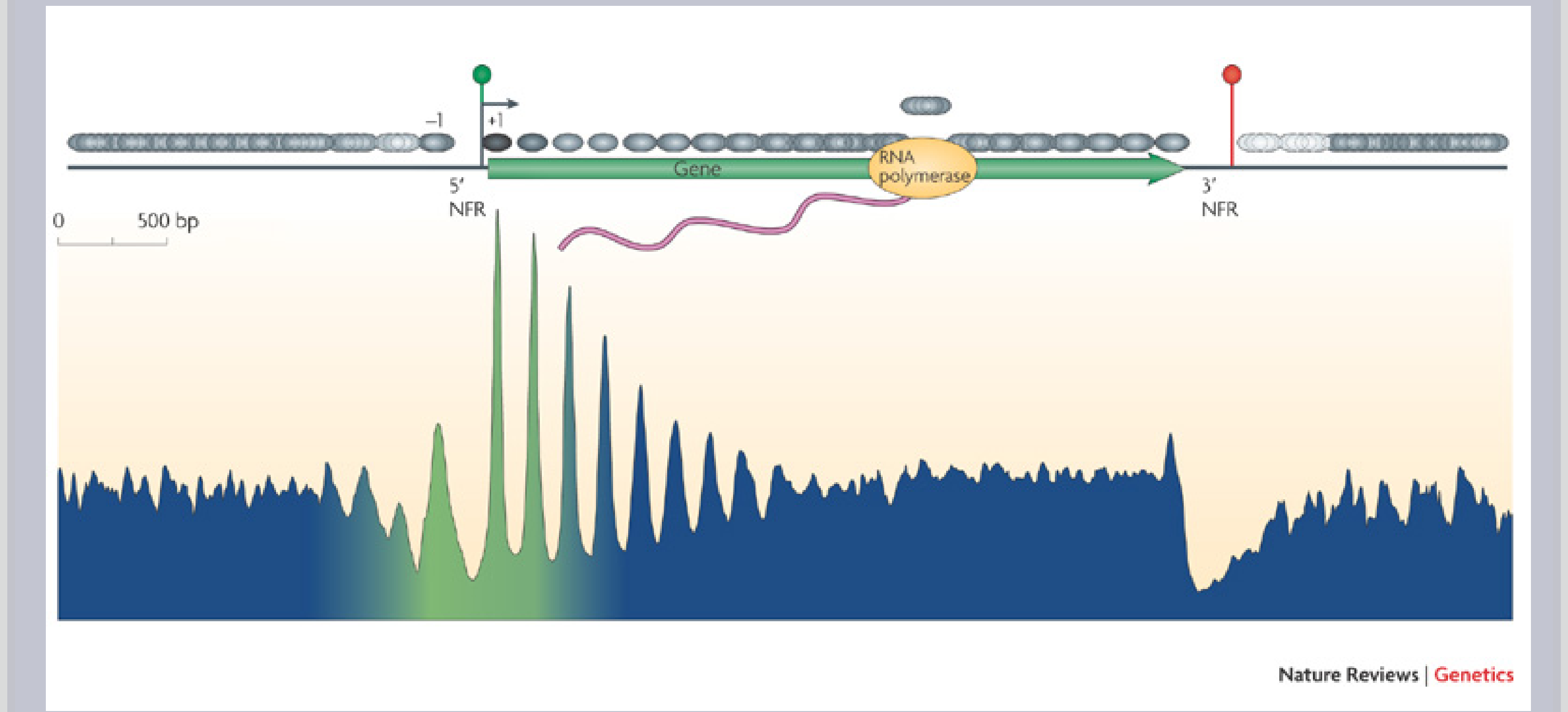


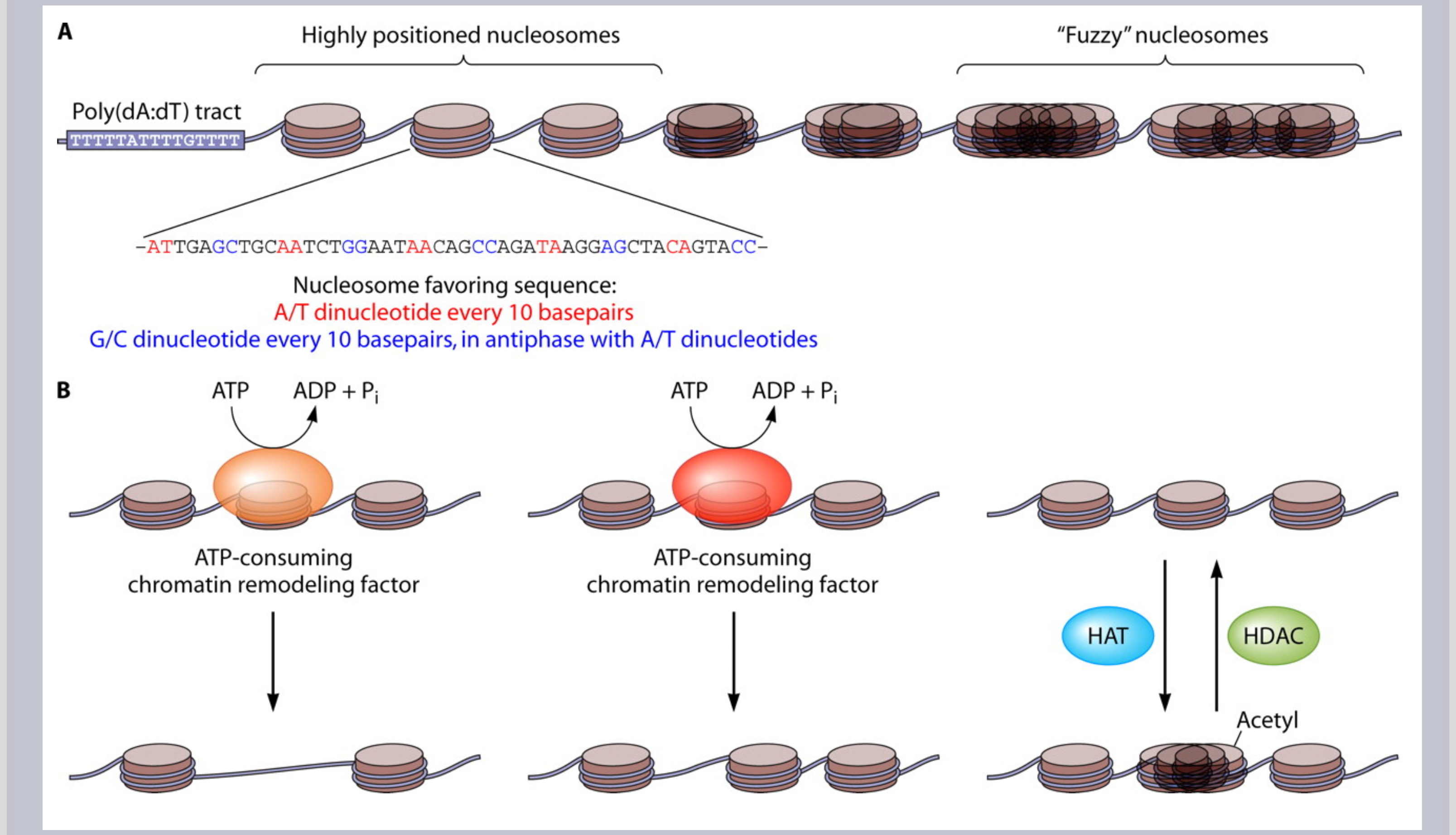
nucleosome position

nucleosome positioning during transcription



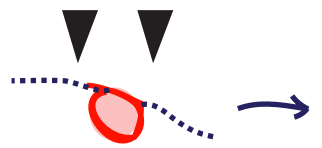
Jiang et al NRG 2009

determinants of positioning stability

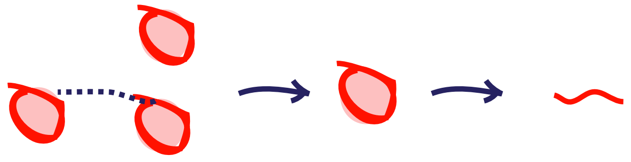

A
Highly positioned nucleosomes
"Fuzzy" nucleosomes
Poly(dA:dT) tract
TTTTTATTTTGTTTT
–ATTGAGCTGCAATCTGGAATAACAGCCAGATAAGGAGCTACAGTACC–
Nucleosome favoring sequence:
A/T dinucleotide every 10 basepairs
G/C dinucleotide every 10 basepairs, in antiphase with A/T dinucleotides
B
ATP
ADP + P$_i$
ATP-consuming chromatin remodeling factor
ATP
ADP + P$_i$
ATP-consuming chromatin remodeling factor
HAT
HDAC
Acetyl

methodology (MNase digestion)

MNase I digests linker DNA, releasing multisomes



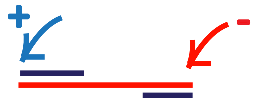
purify mononucleosome-bound DNA



library construction and sequencing

mapping to genome reference (BWA or bowtie2)

identify cleavage location

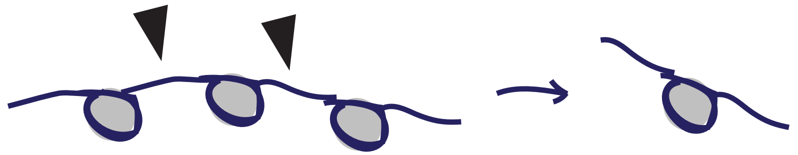


calculate nucleosomal density

call positioned nucleosomes

methodology (transposon insertion)

Tn5 integrates into accessible chromatin, and releases multisomes



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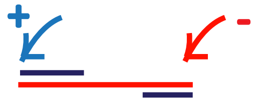
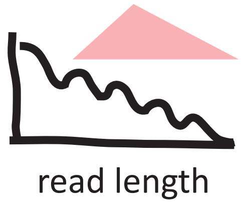


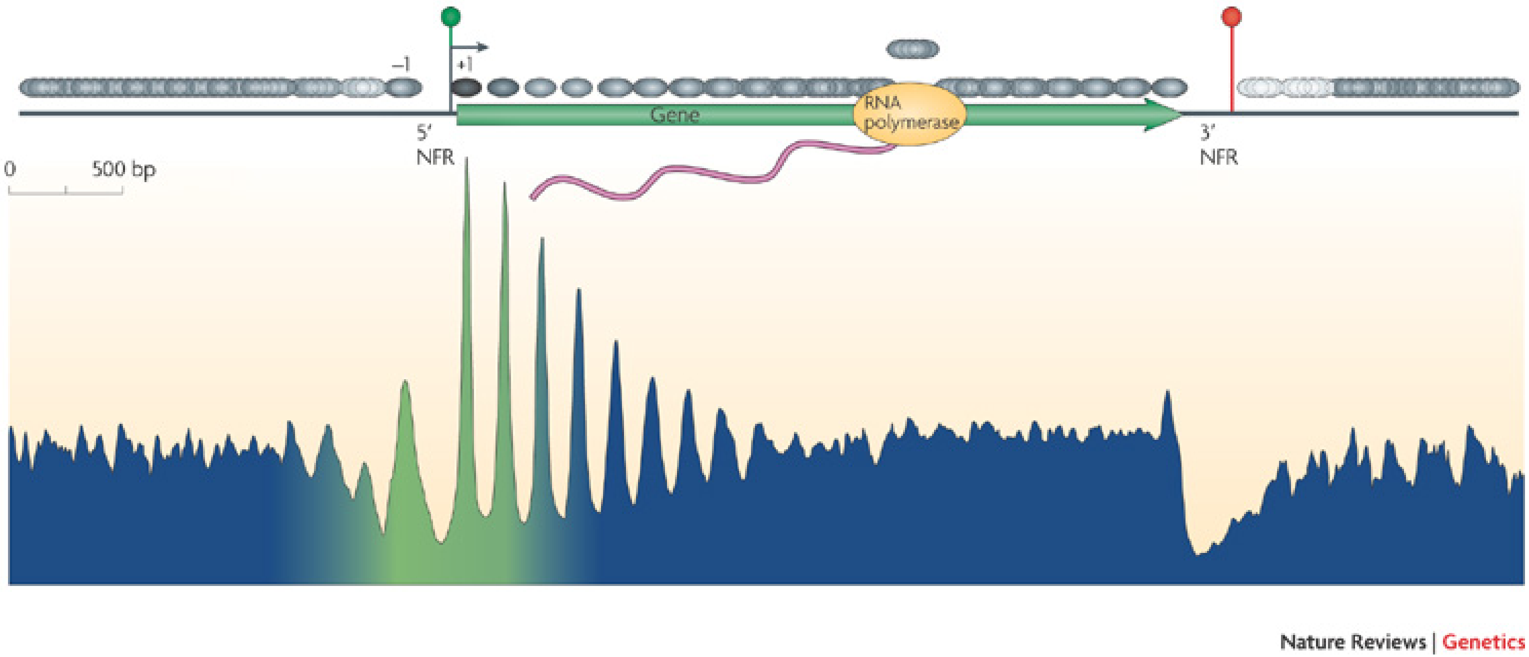
identify reads that span at least one nucleosome



calculate density of tags / number of insertion events

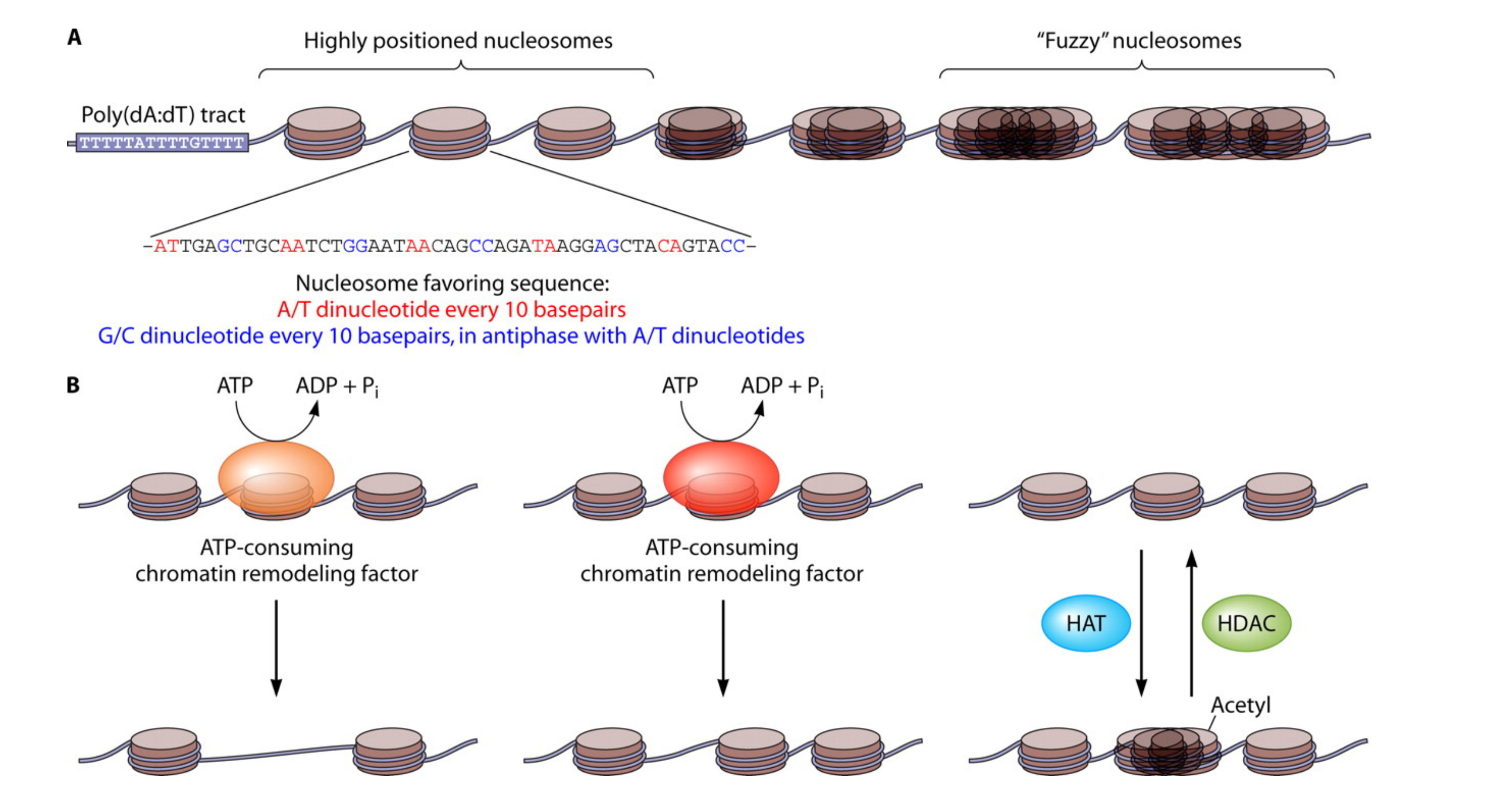
call positioned nucleosomes

nucleosome positioning during transcription



Jiang et al NRG 2009

positioning stability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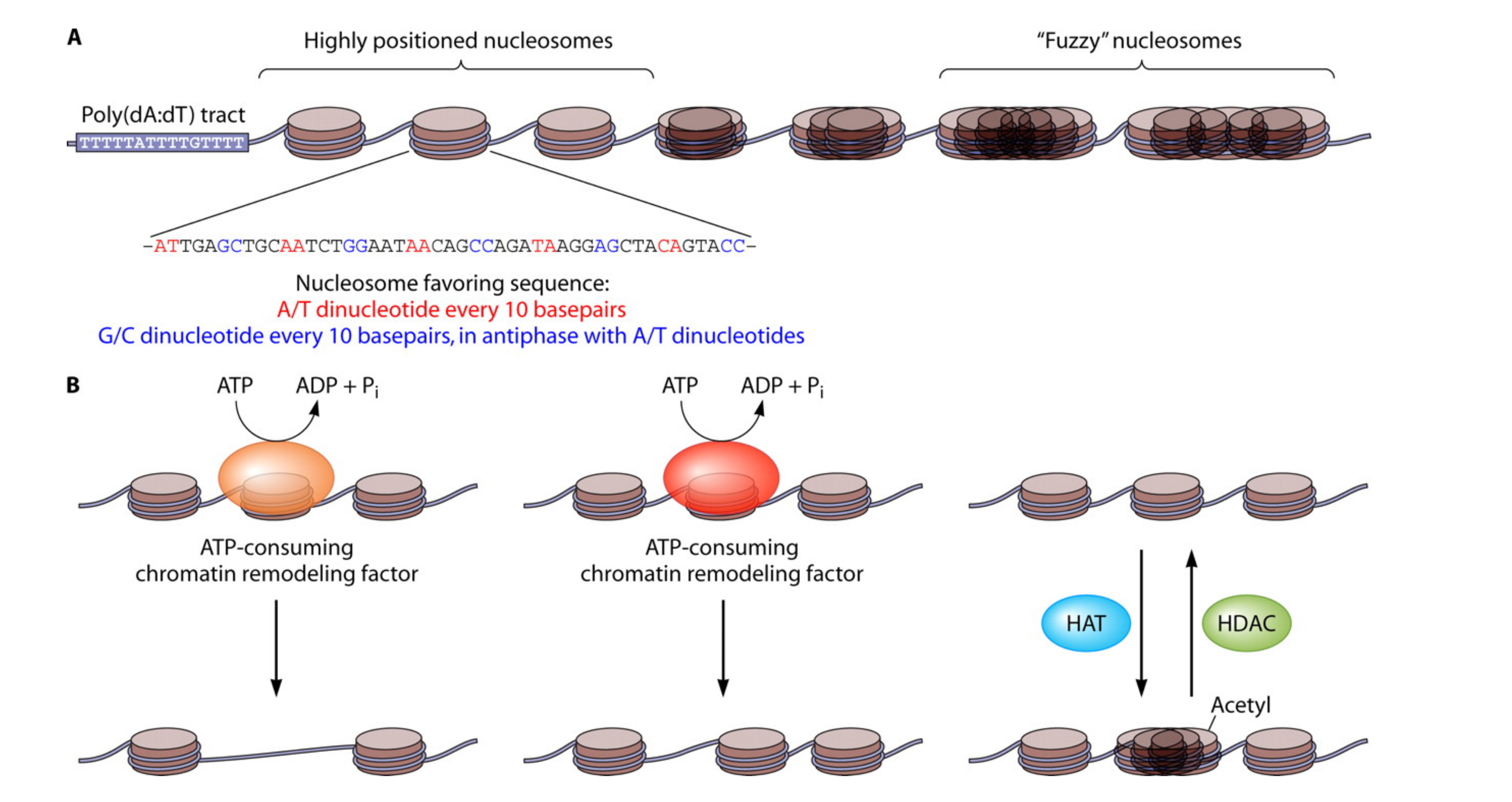
Acetyl

positioning stability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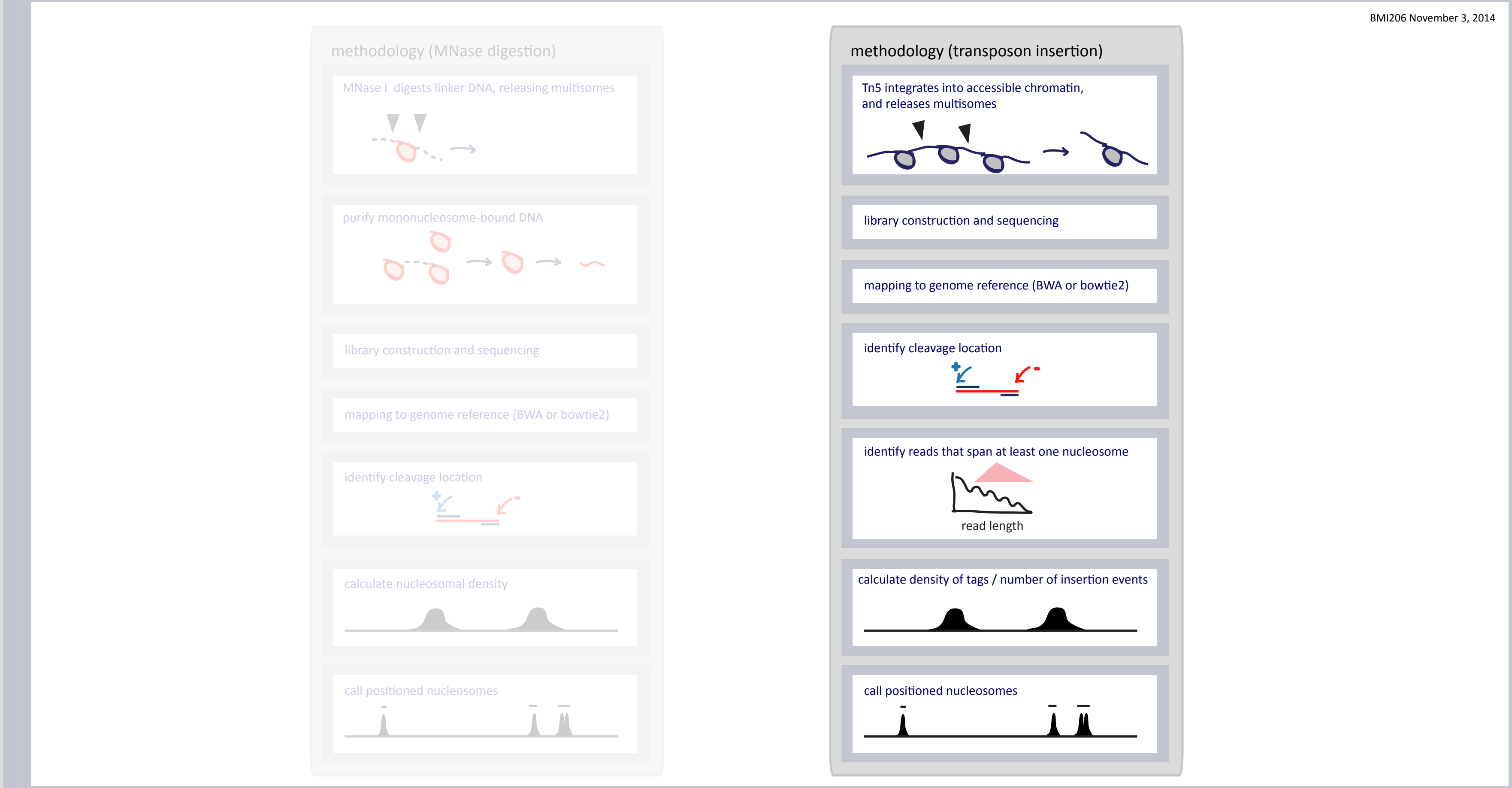
ATP
ADP + P$_i$
ATP
ADP + P$_i$
ATP-consuming
chromatin remodeling factor
ATP-consuming
chromatin remodeling factor
HAT
HDAC
Acetyl

two common methodologies (MNase, ATAC-seq)



BMI206 November 3, 2014

methodology (MNase digestion)

MNase I digests linker DNA, releasing multisomes

purify mononucleosome-bound DNA

library construction and sequencing

mapping to genome reference (BWA or bowtie2)

identify cleavage location

calculate nucleosomal density

call positioned nucleosomes

methodology (transposon insertion)

Tn5 integrates into accessible chromatin, and releases multisomes

library construction and sequencing

mapping to genome reference (BWA or bowtie2)

identify cleavage location

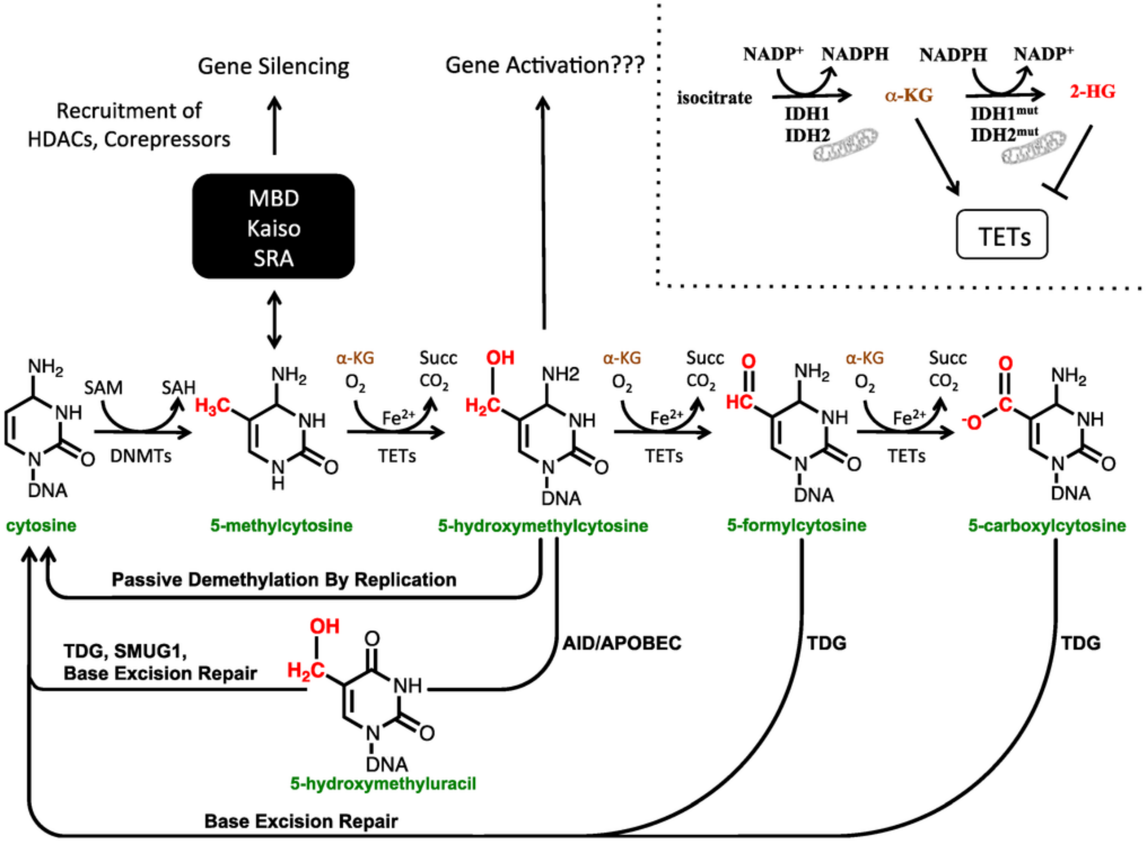
identify reads that span at least one nucleosome

read length

calculate density of tags / number of insertion events

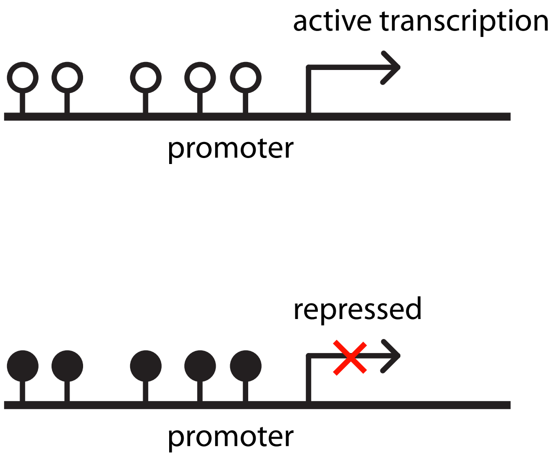
call positioned nucleosomes

catalogue of common base modifications (in mammals)



Mariani et al, Cancers 2013

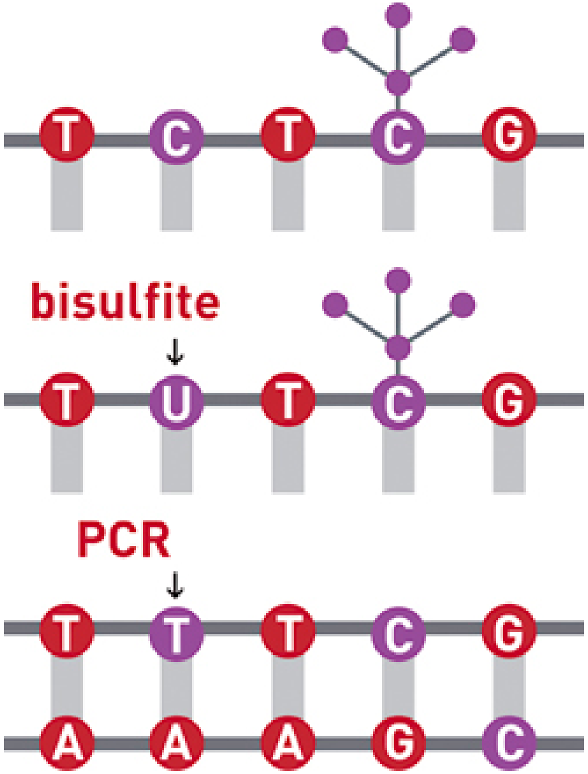
5mC repression of gene expression



● 5mC 5 methyl cytosine

○ C unmodified cytosine

bisulfite conversion / protection



daigenode.com

methodology

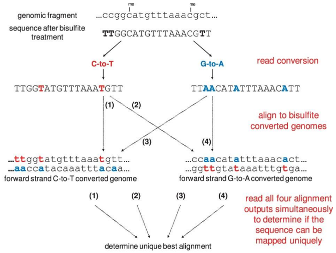
convert DNA with bisulfite

methyl CGTTCG → CGTTUG

fragmentation

library construction and sequencing

mapping


me
me
genomic fragment
...ccggcatgtttaaacgct...
sequence after bisulfite treatment
TTGGCATGTTTAAACGTT
C-to-T
G-to-A
read conversion
TTGGTATGTTTAAATGTT
TTAACATATTTAAACATT
(1)
(2)
align to bisulfite converted genomes
(3)
(4)
...ttggtatgtttaaatgtt...
...aaccatacaaatttacaa...
forward strand C-to-T converted genome
...ccaacatatttaaacact...
...ggttgtataaatttgtga...
forward strand G-to-A converted genome
(1)
(2)
(3)
(4)
read all four alignment outputs simultaneously to determine if the sequence can be mapped uniquely
determine unique best alignment

Krueger et al Bioinformatics 2011

calculate % of tags methylated per genomic position

methodology

convert DNA with bisulfite

methyl CGTTCG → CGTTUG

fragmentation

library construction and sequencing

methodology

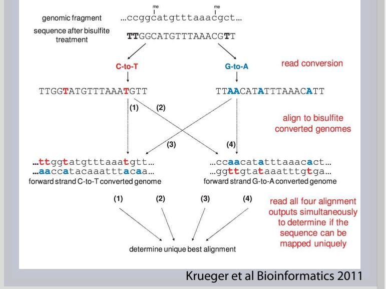
convert DNA with bisulfite

meth
CGTTCG
CGTTUG

fragmentation

library construction and sequencing

mapping

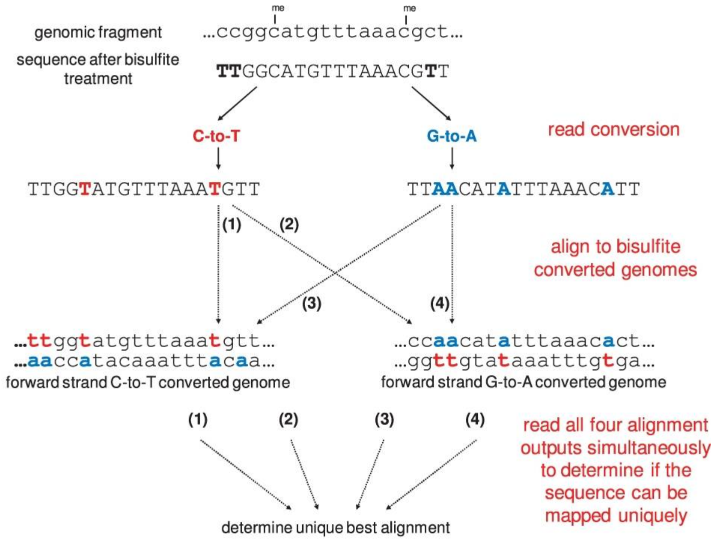


Krueger et al Bioinformatics 2011

calculate % of tags methylated per genomic position

methodology

mapping


me
me
genomic fragment
...ccggcatgtttaaacgct...
sequence after bisulfite treatment
TTGGCATGTTTAAACGTT
C-to-T
G-to-A
read conversion
TTGGTATGTTTAAATGTT
TTAACATATTTAAACATT
(1)
(2)
(3)
(4)
align to bisulfite converted genomes
...ttggtatgtttaaatgtt...
...aaccatacaaatttacaa...
forward strand C-to-T converted genome
...ccaacatatttaaacact...
...ggttgtataaatttgtga...
forward strand G-to-A converted genome
(1)
(2)
(3)
(4)
read all four alignment outputs simultaneously to determine if the sequence can be mapped uniquely
determine unique best alignment

Krueger et al Bioinformatics 2011

calculate % of tags methylated per genomic position

GEM (Guo et al, PLoS Comp. Bio 2012)

algorithm

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2. discover set of enriched kmers at binding event
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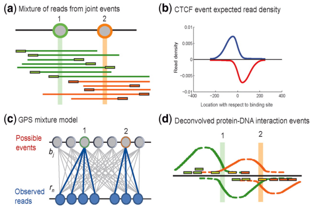
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Observed reads
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Guo et al, Bioinformatics 2010

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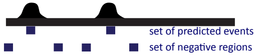

set of predicted events from (1)

set of negative regions (+/-300bp)

	positive region count	negative region count
kmer1	400	34
kmer2	25	42
kmer...	17	25

hypergeometric test

Barash et al WABI 2001

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6. Redo steps (2) and (3) to improve motif quality

http://www.ploscompbiol.org/article/info%3Adoi%2F10.1371%2Fjournal.pcbi.1002638

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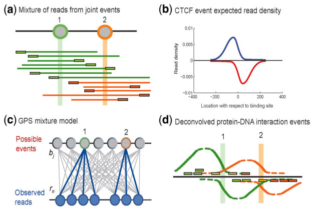
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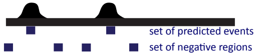

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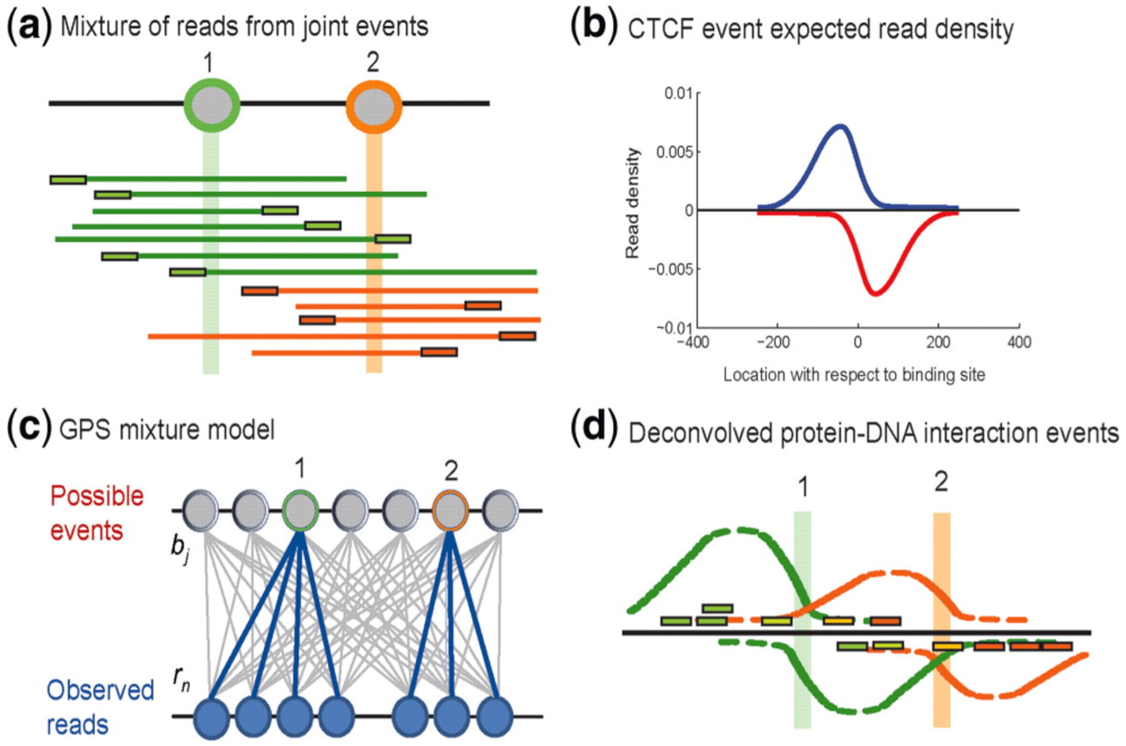
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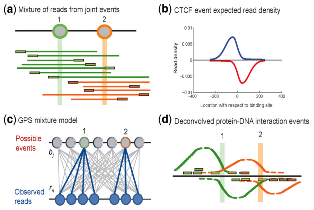
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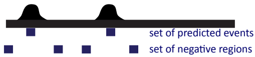

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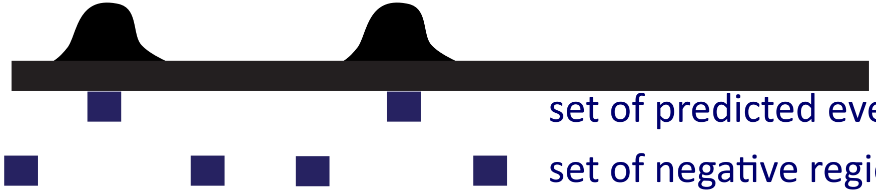

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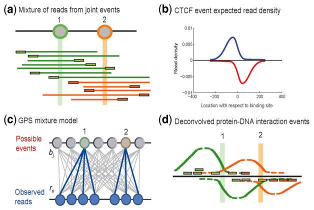
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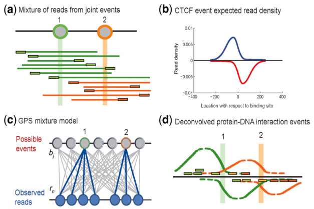
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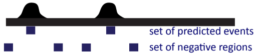
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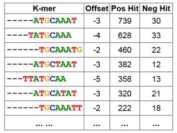

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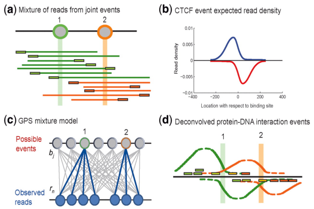
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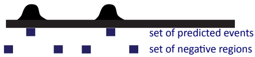

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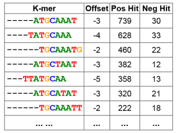

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