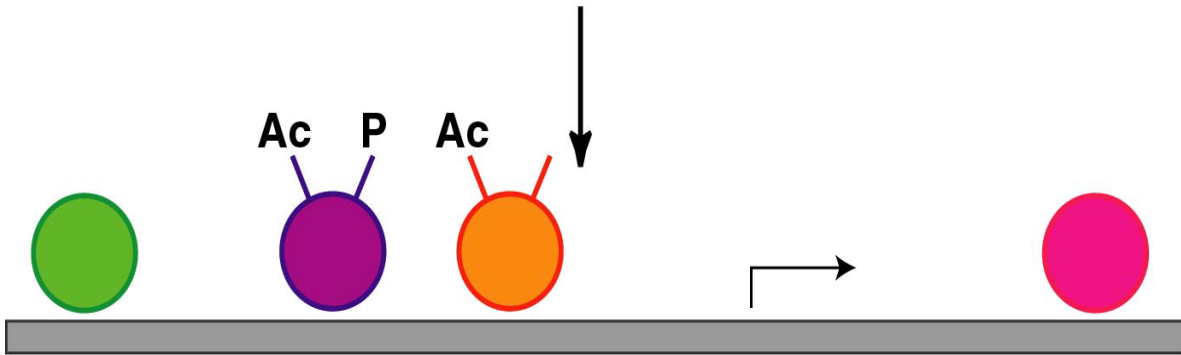
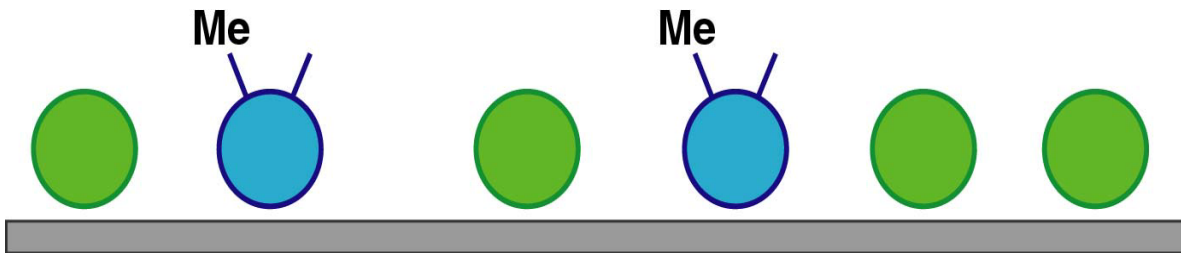
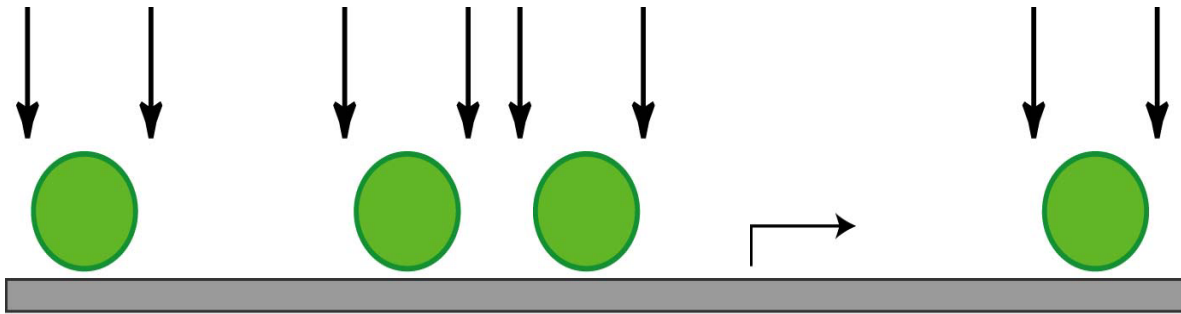
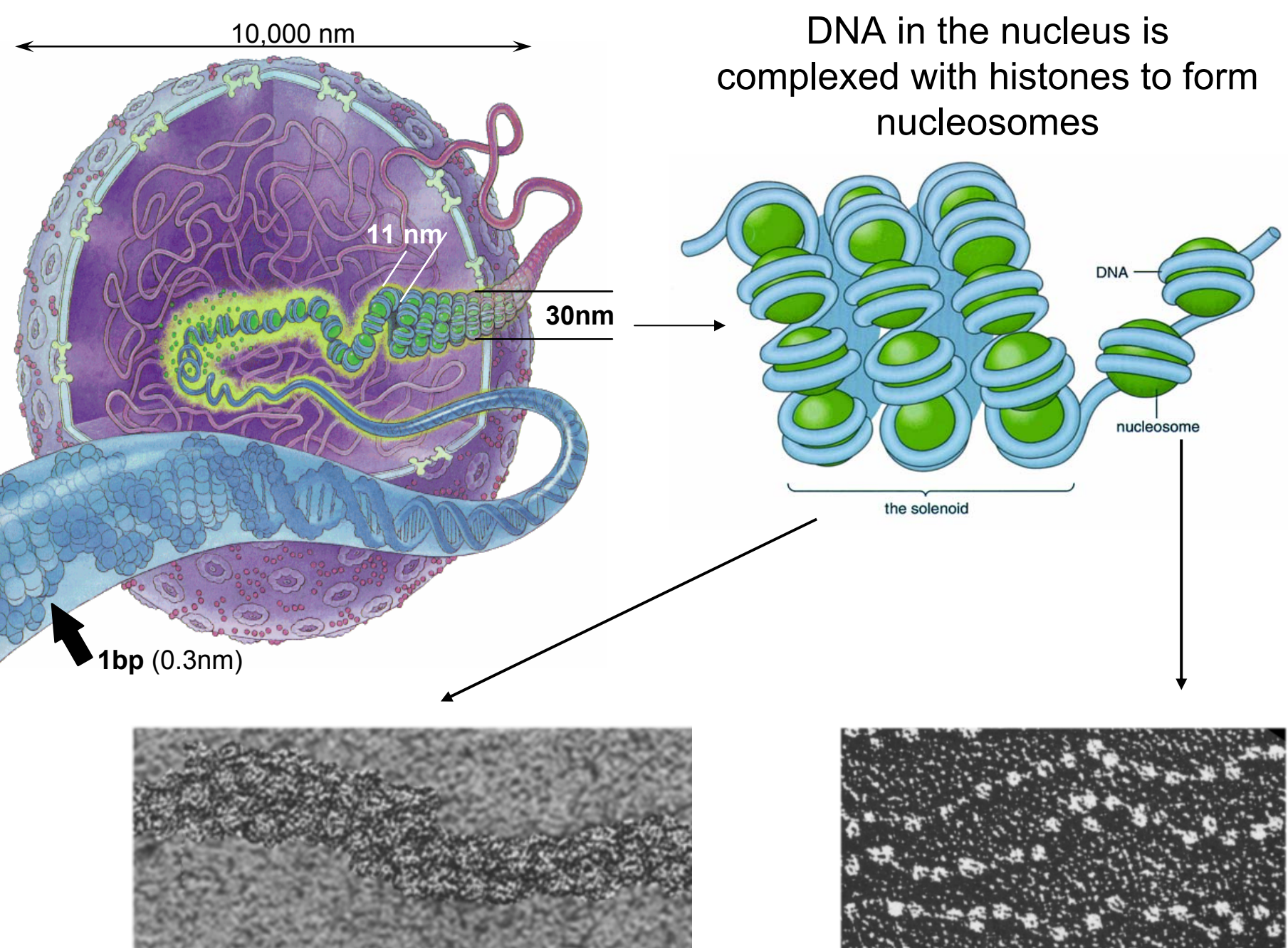
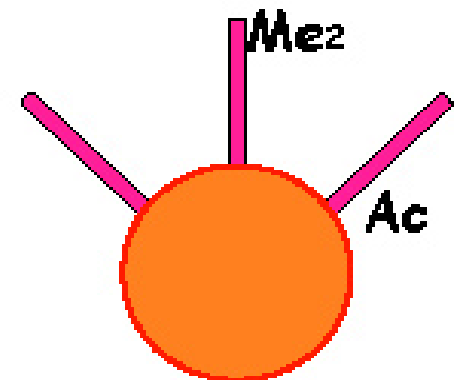
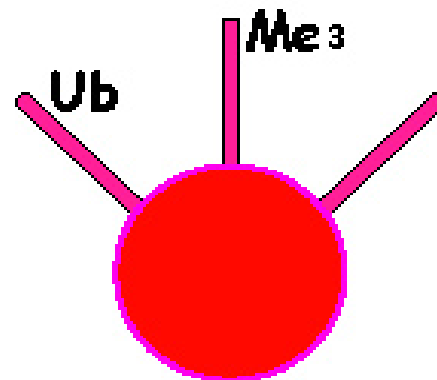
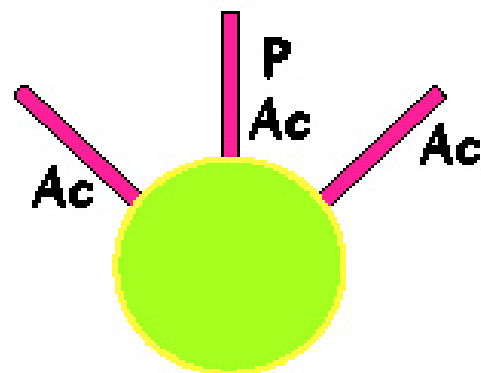
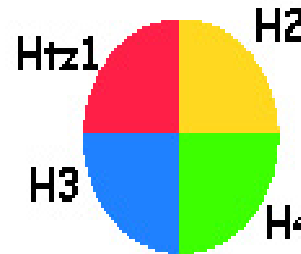
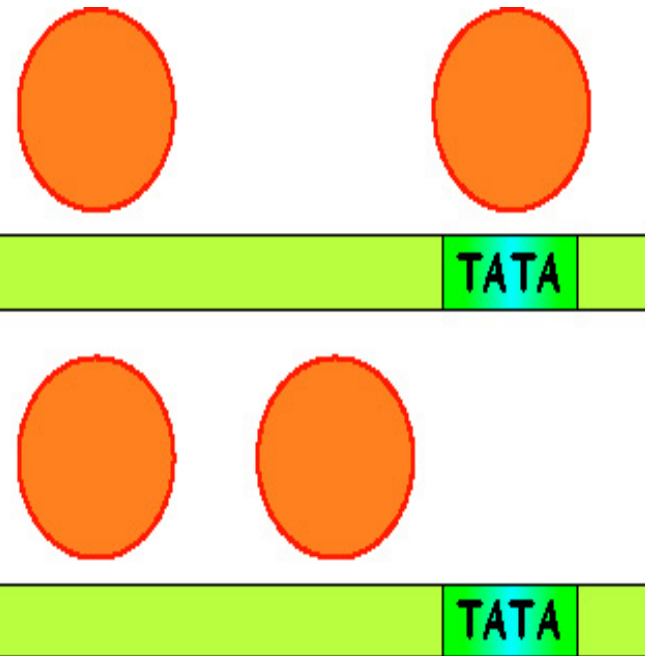




Array-based
approaches
to chromatin
structure and
function



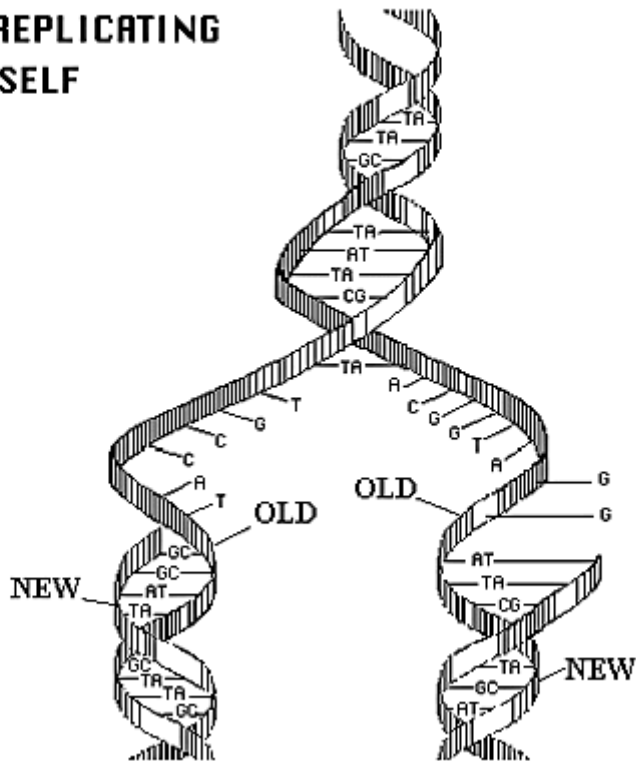
Primary structure of chromatin consists of:



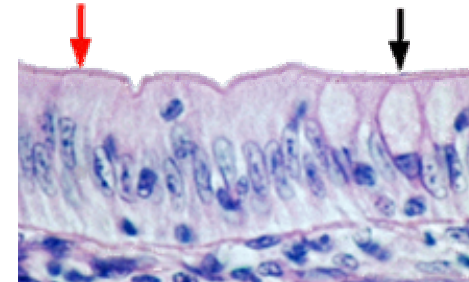
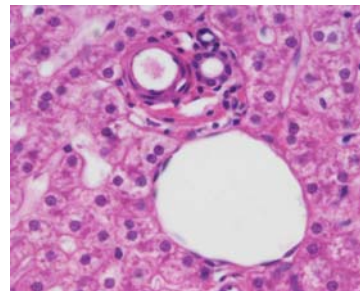
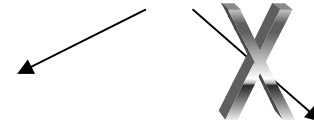
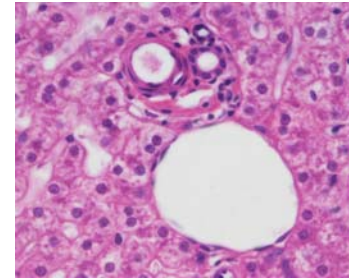
How do cells pass non-DNA information on to their daughters?

Replication of DNA is well understood (relatively)

DNA REPLICATING ITSELF



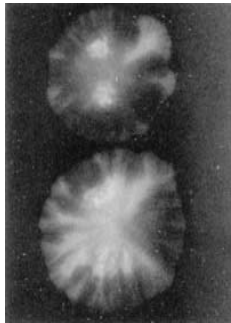
Despite sharing a genome with intestinal cells, when liver cells divide they always make more liver cells. This is an example of “epigenetic” inheritance



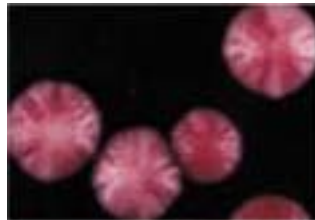
How does this work?

Chromatin states can also be inherited!

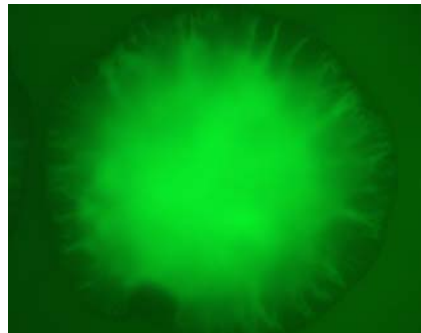
SIR2-dependent repression of
subtelomeric DNA is metastably heritable:



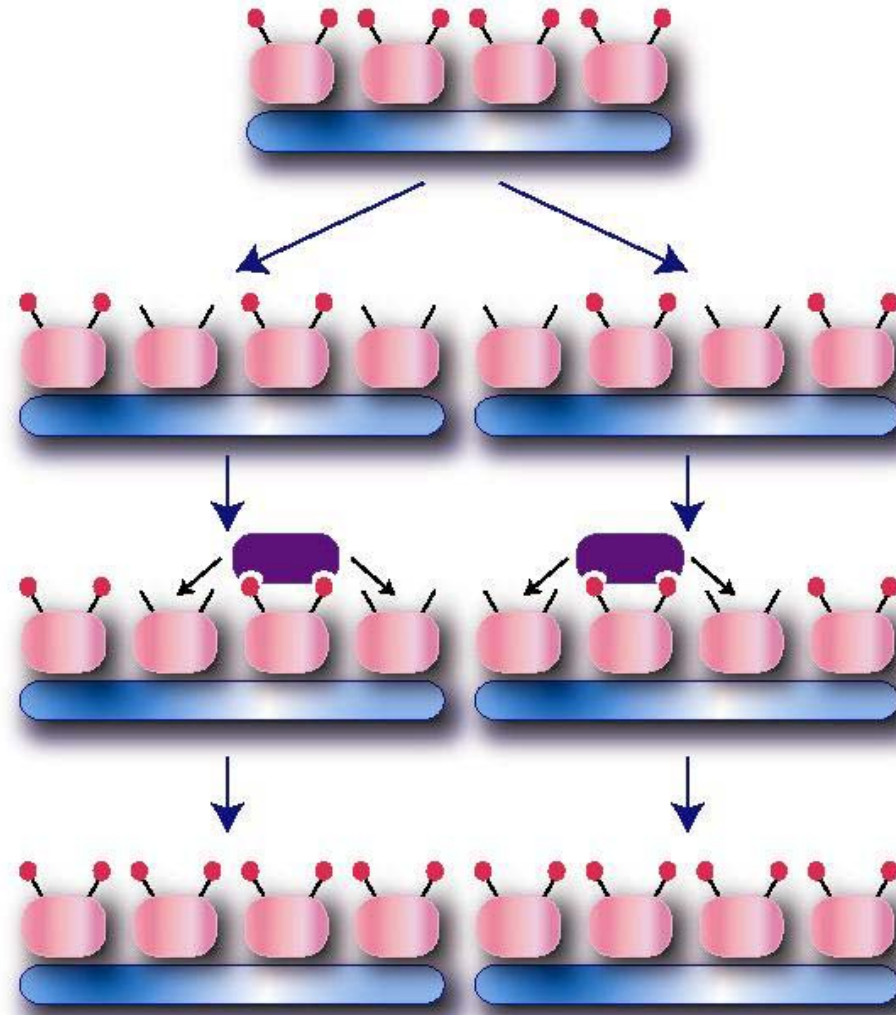
Gottschling et al., Cell, 1990



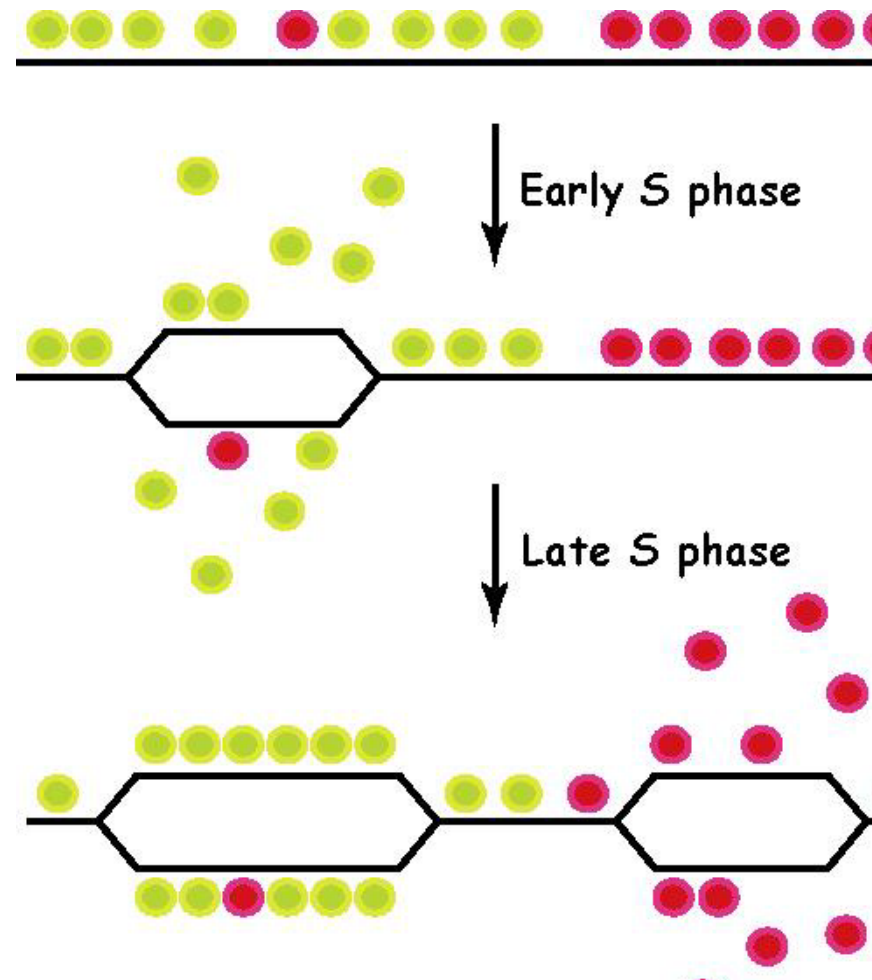
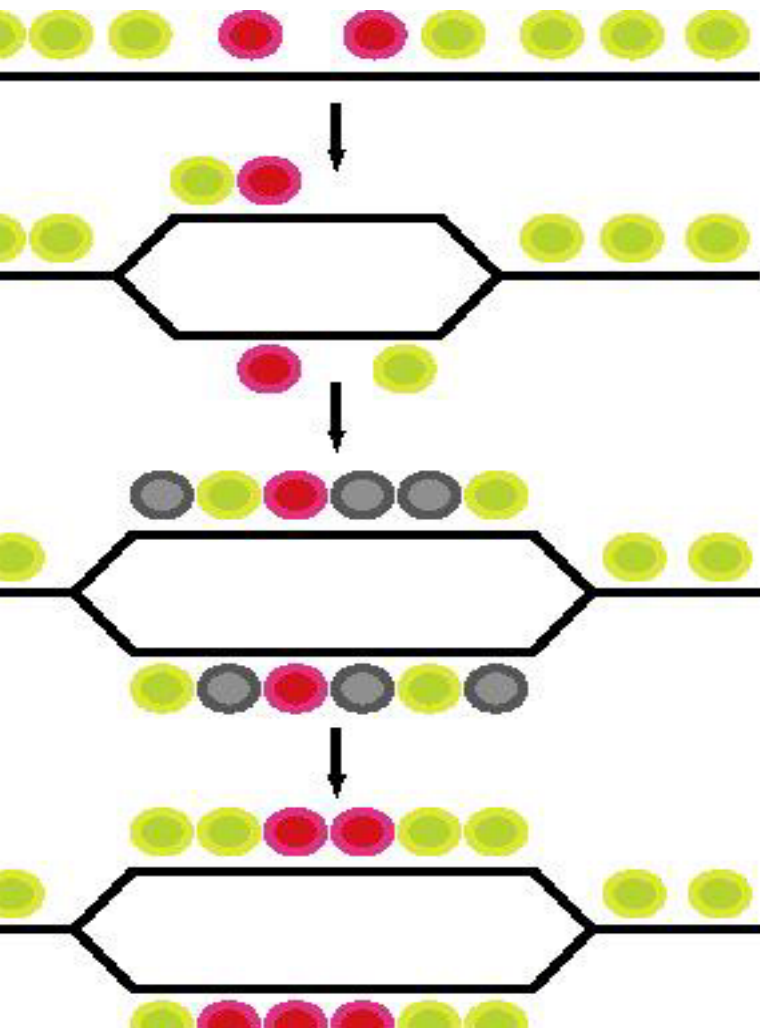
Zhang et al., Nature, 2000



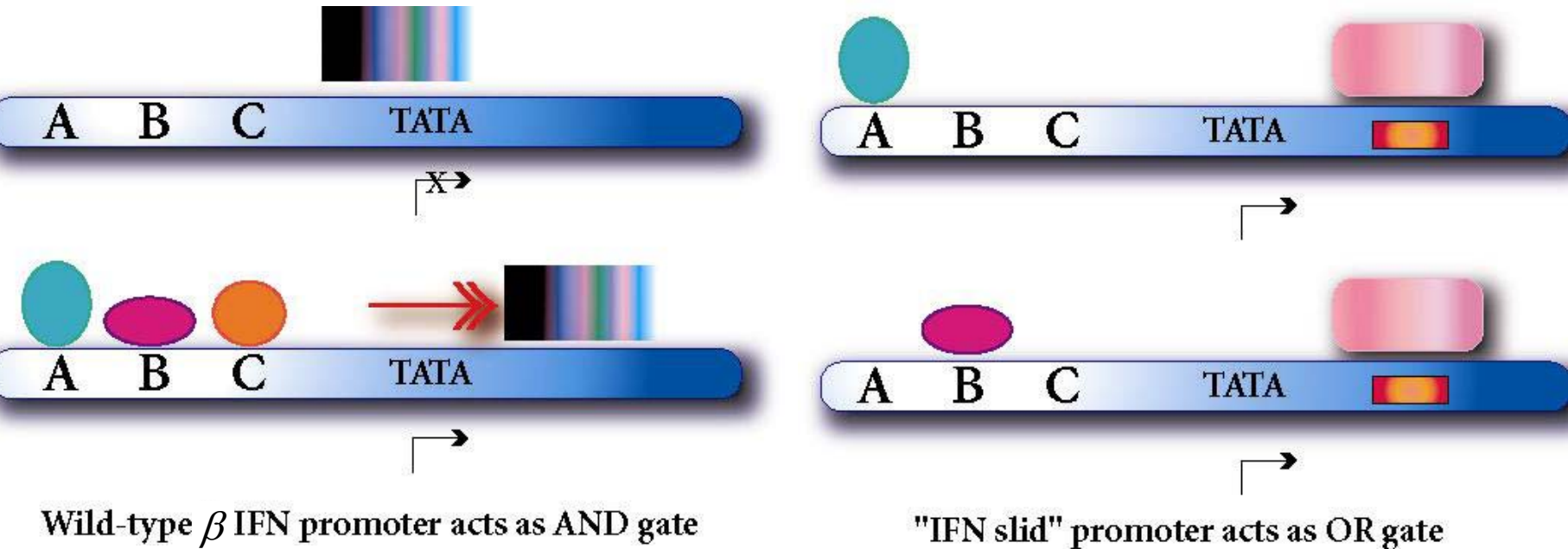
Dion et al. the future



“Timing” and “distribution” models for inheritance of chromatin state

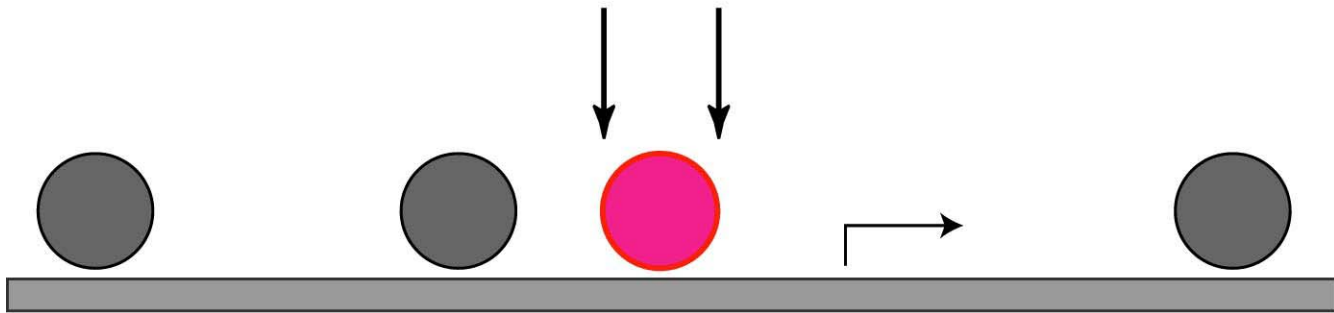
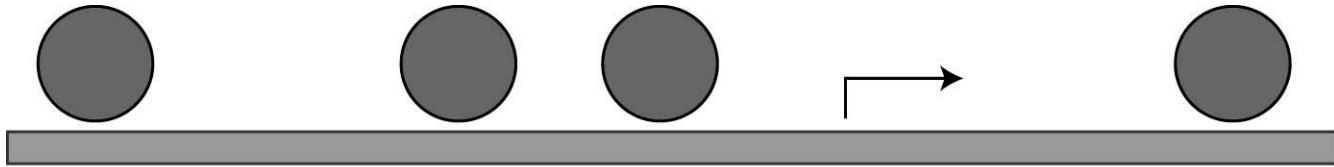


Chromatin is involved in signal integration at the transcriptional level

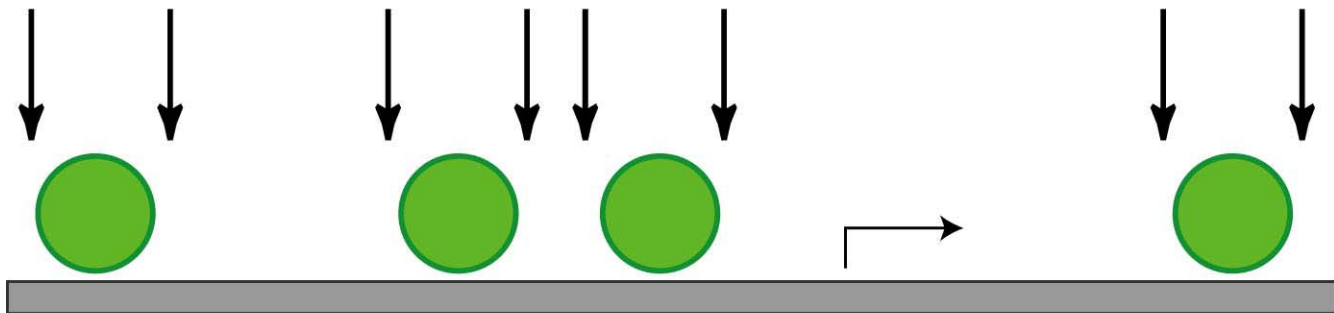


The position of the nucleosome changes the regulatory logic (and timing) of the promoter!

Nucleosome Positioning

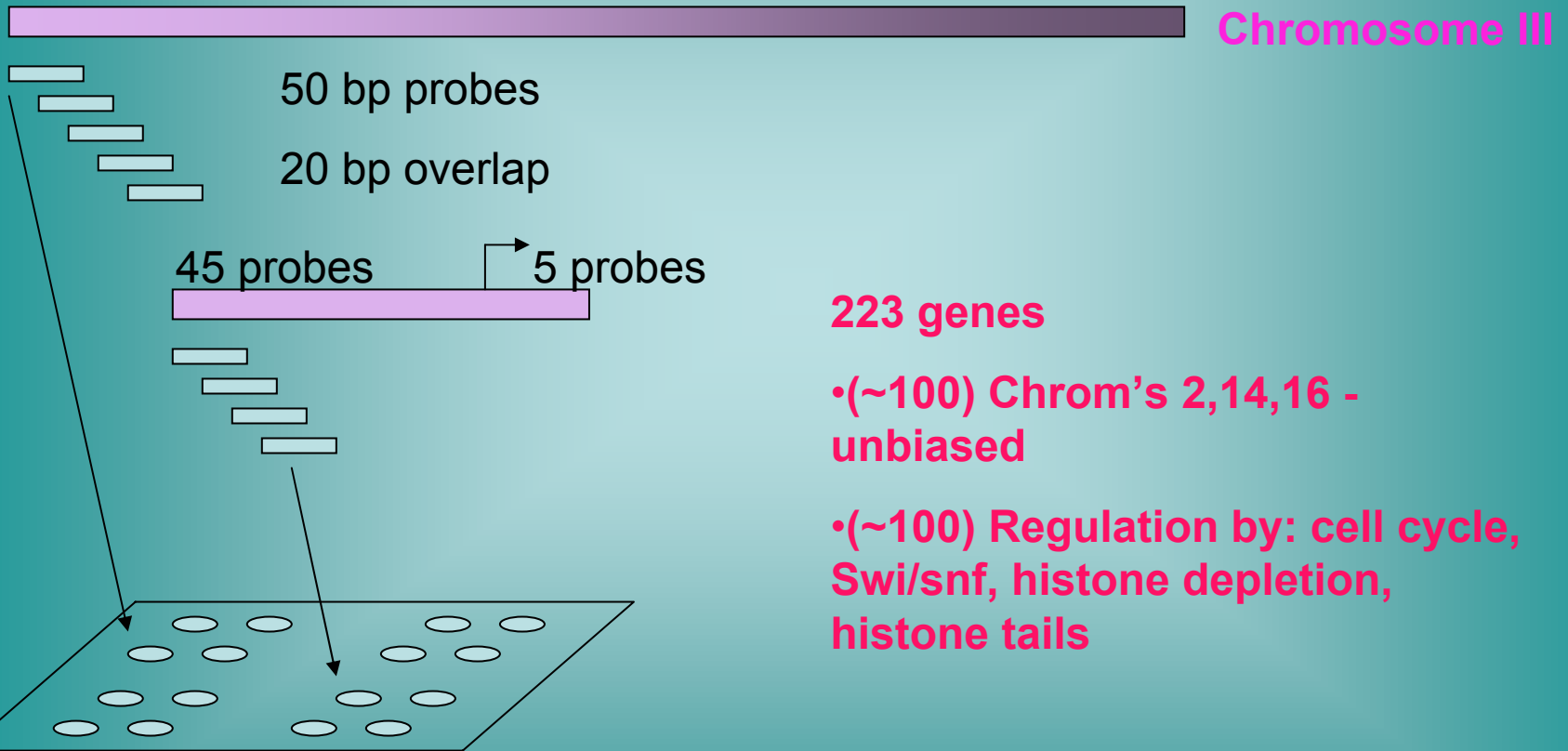


Traditional Methods of Chromatin Analysis

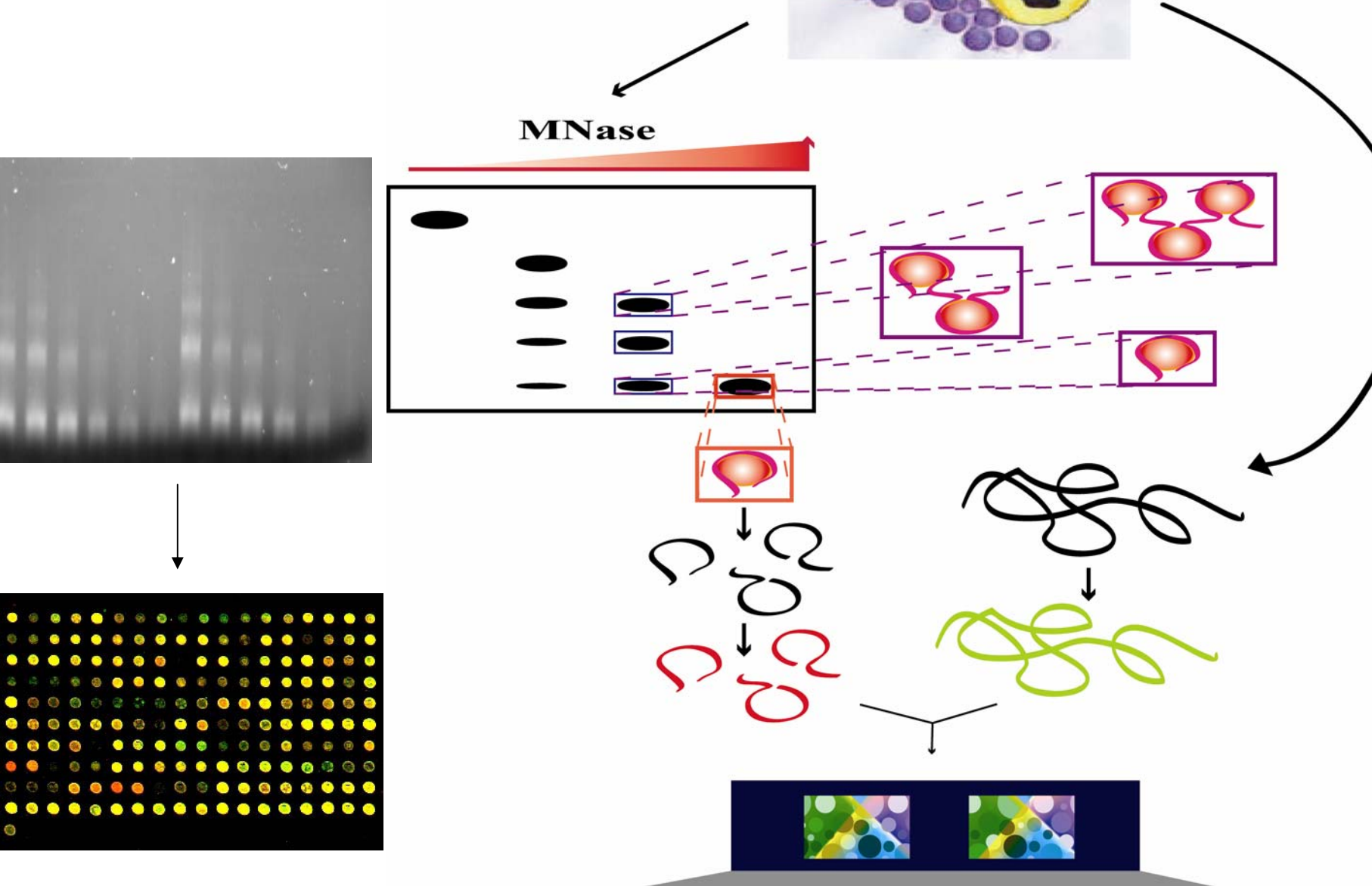


Large Scale Analysis of Chromatin Structure

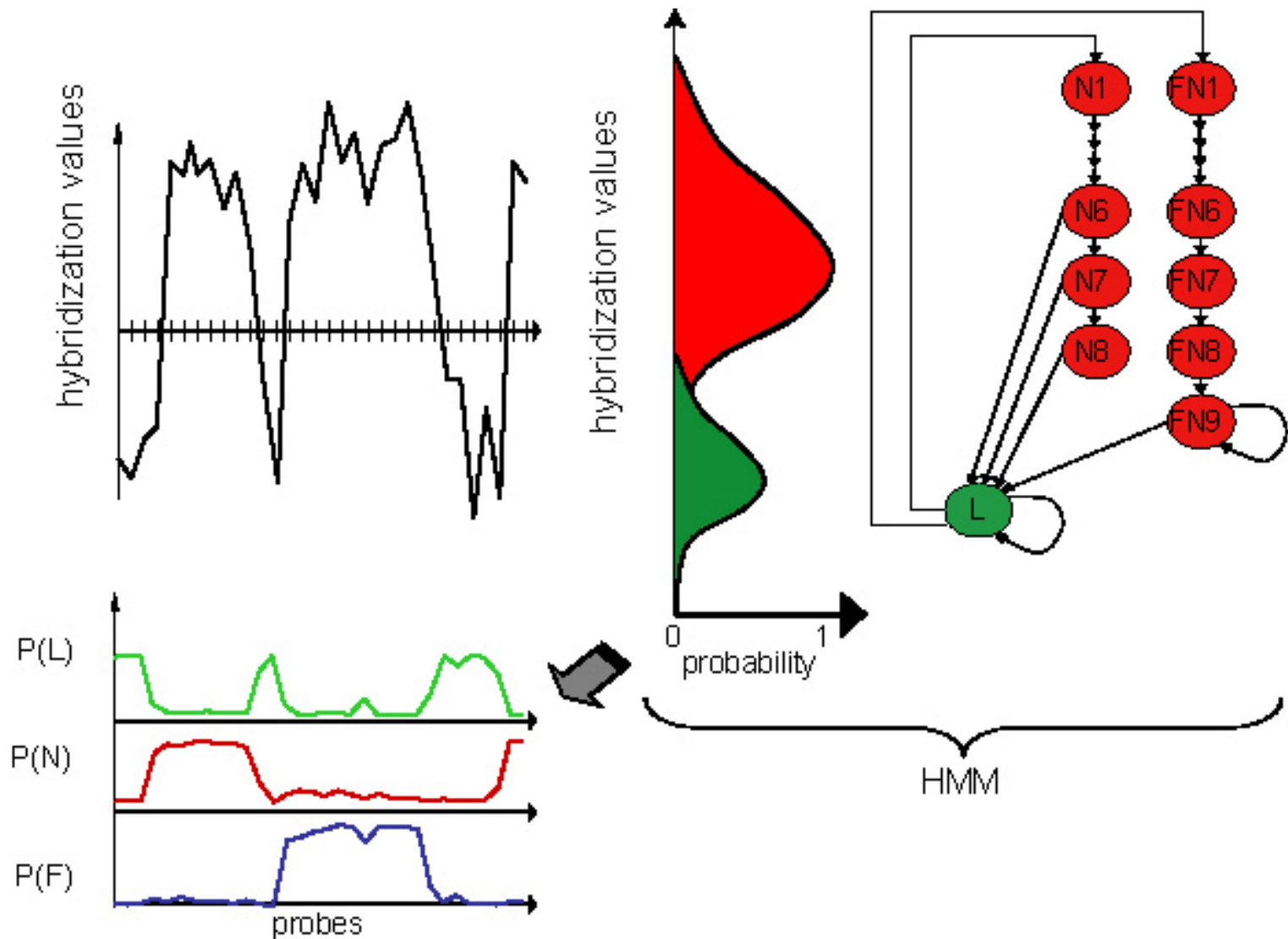
Microarray Design



Strategy part deux



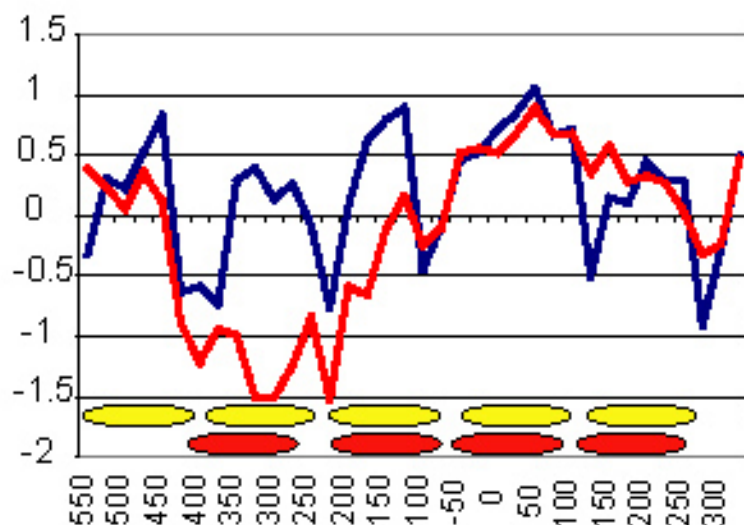
A Hidden Markov Model objectively identifies nucleosome positions



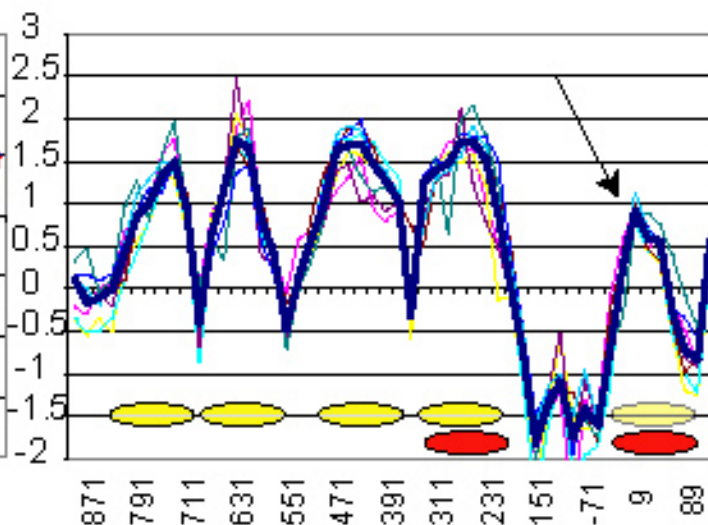
Verification

Mat α

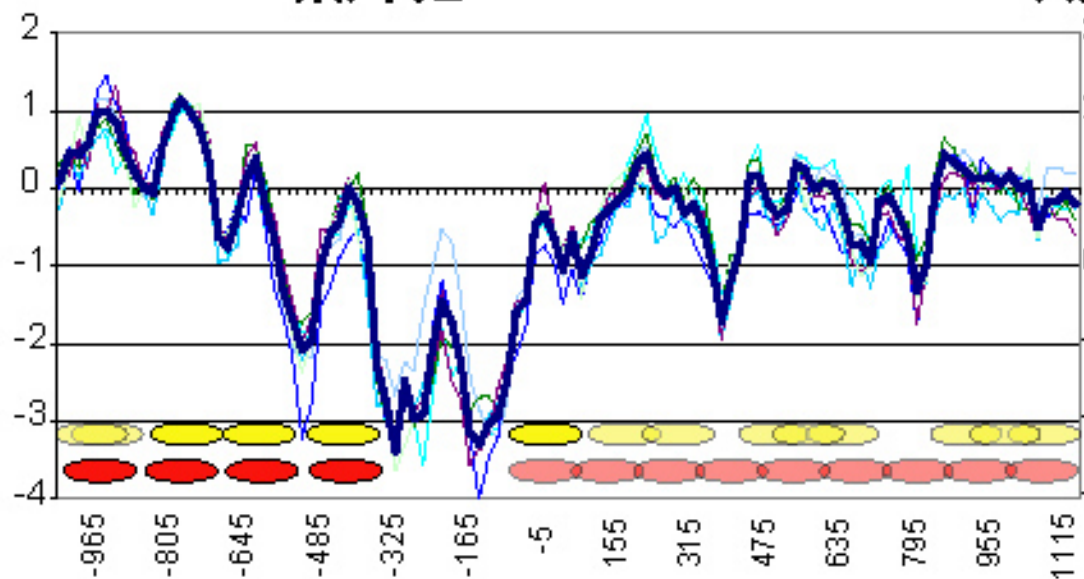
Mat α



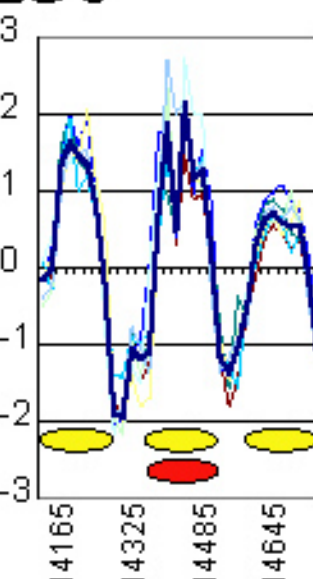
MFA2



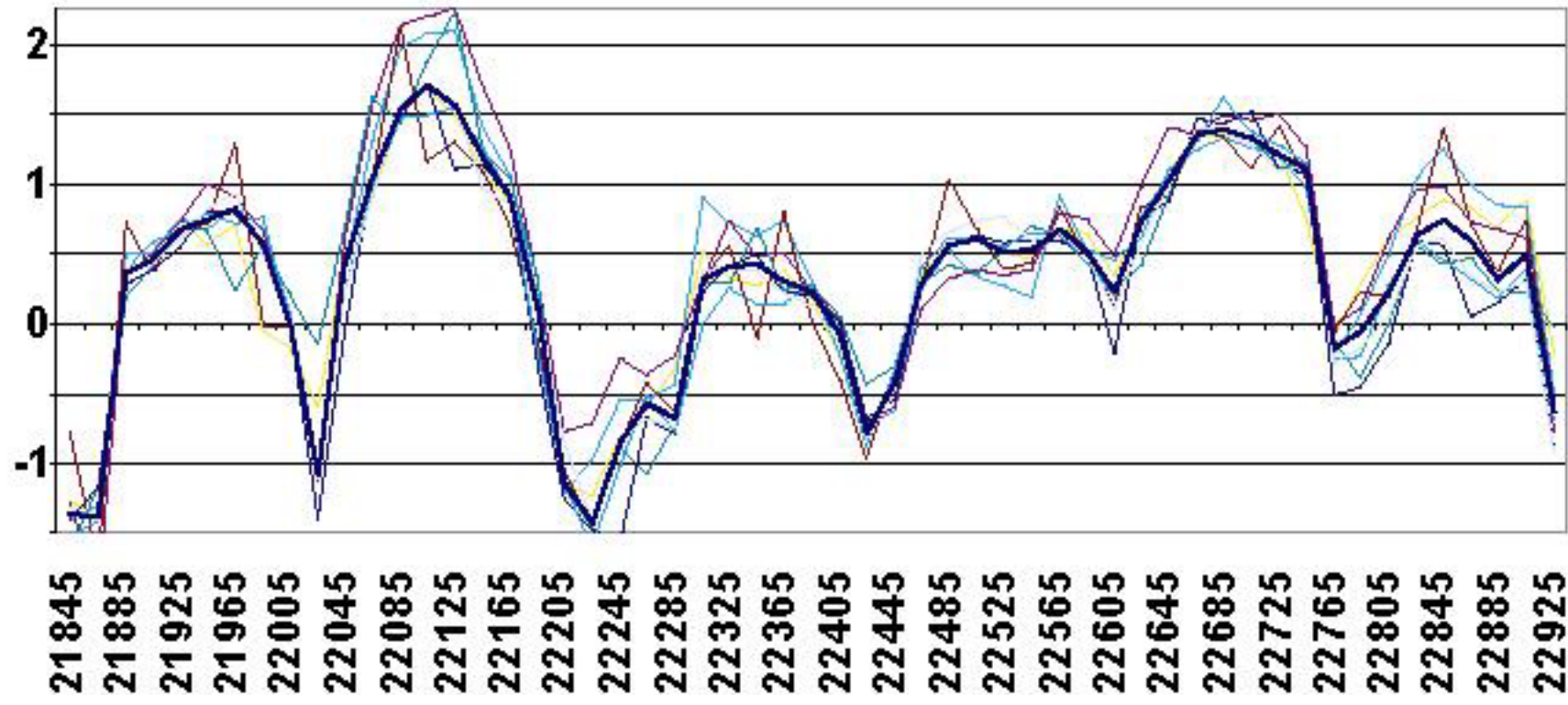
HIS3

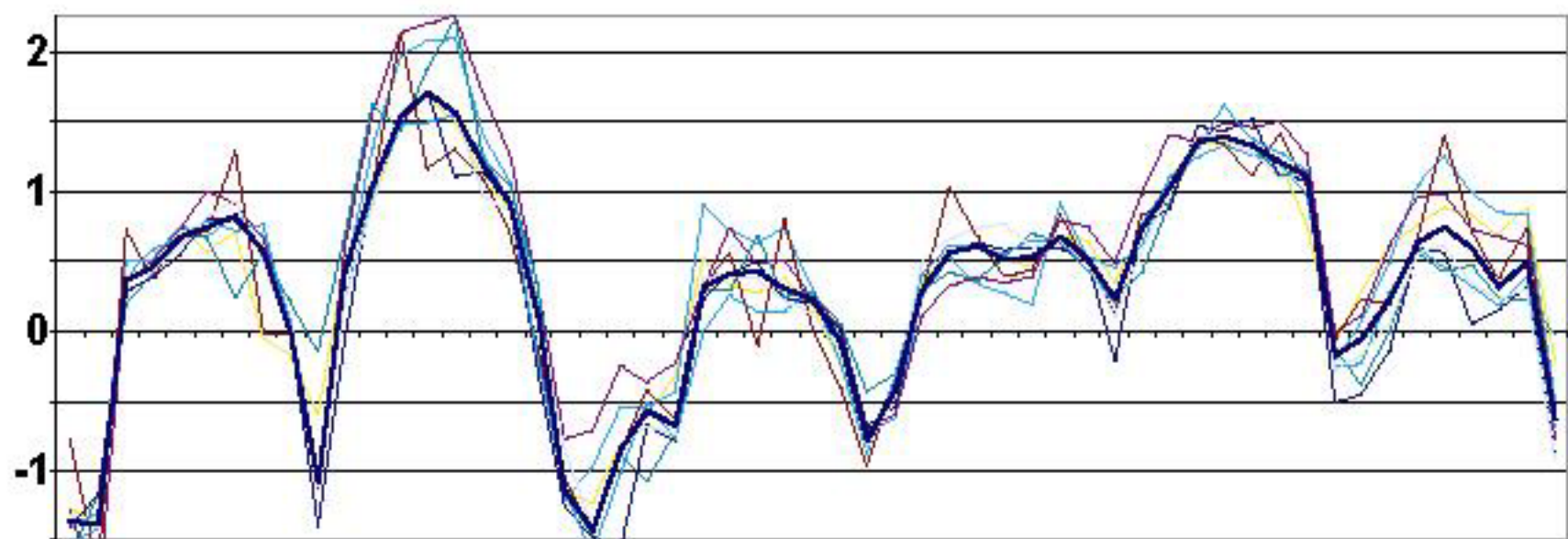


CHA1

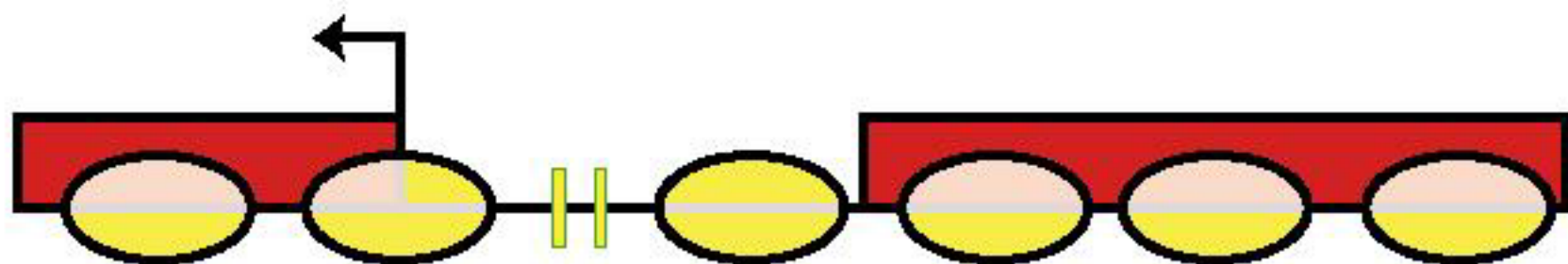


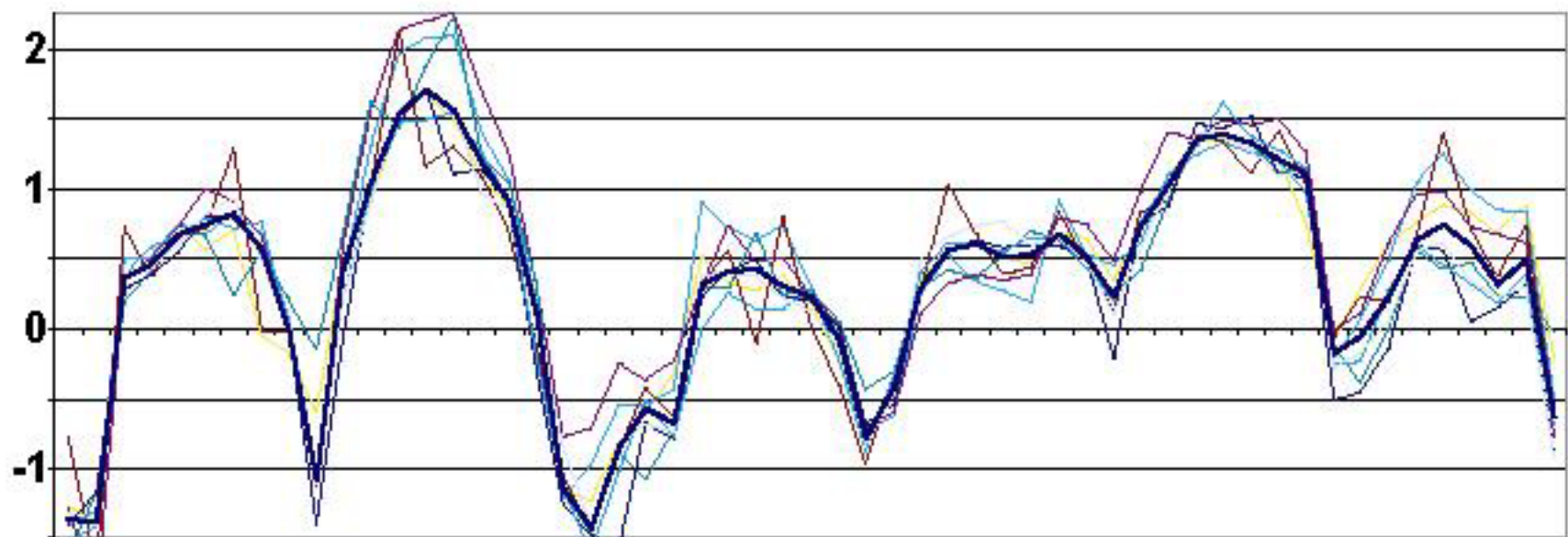
CerIII



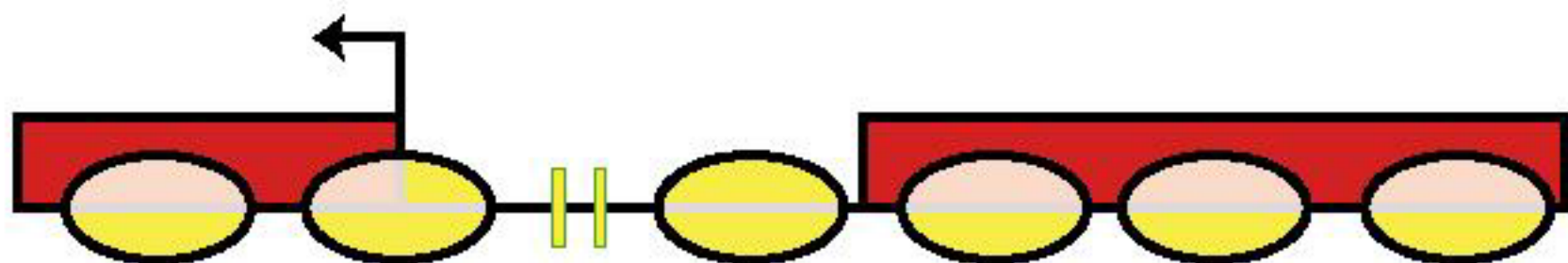


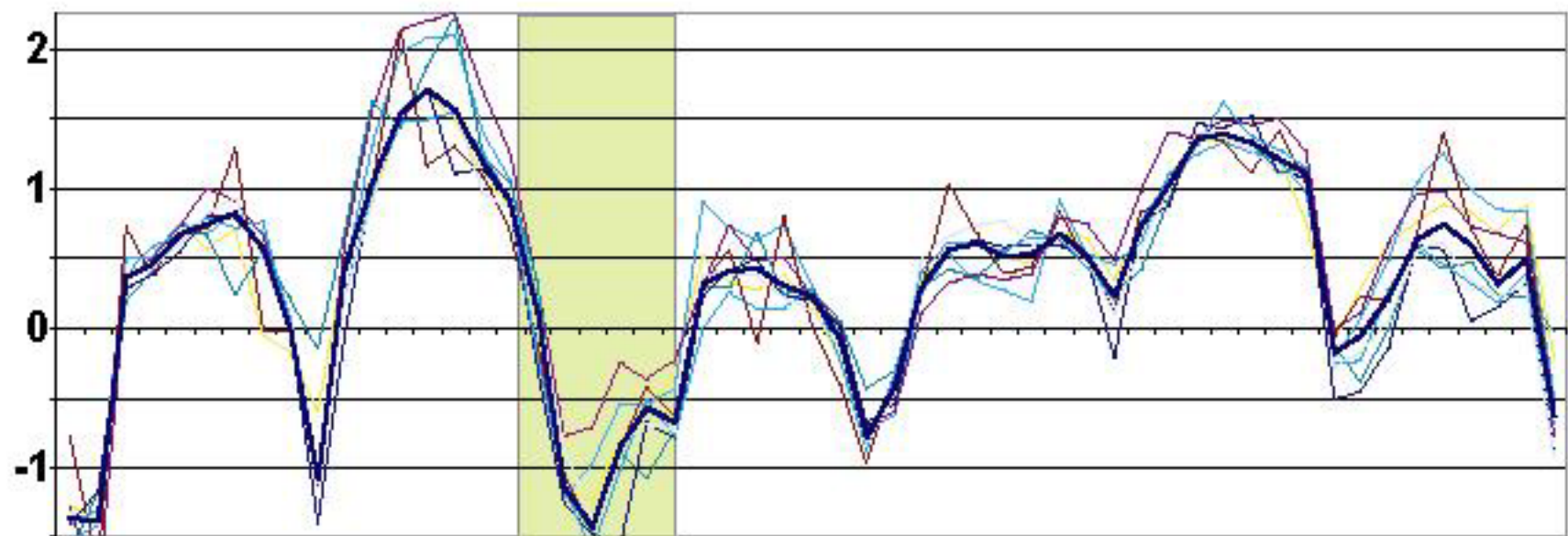
21845 21885 21925 21965 22005 22045 22085 22125 22165 22205 22245 22285 22325 22365 22405 22445 22485 22525 22565 22605 22645 22685 22725 22765 22805 22845 22885 22925



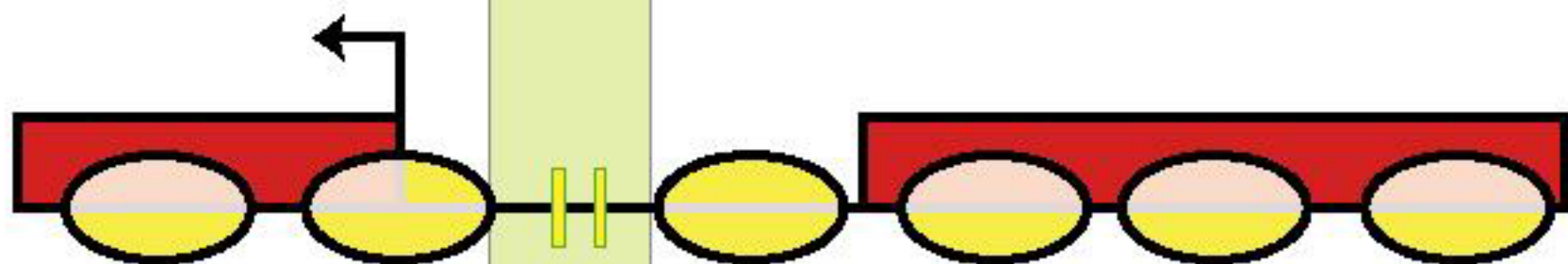


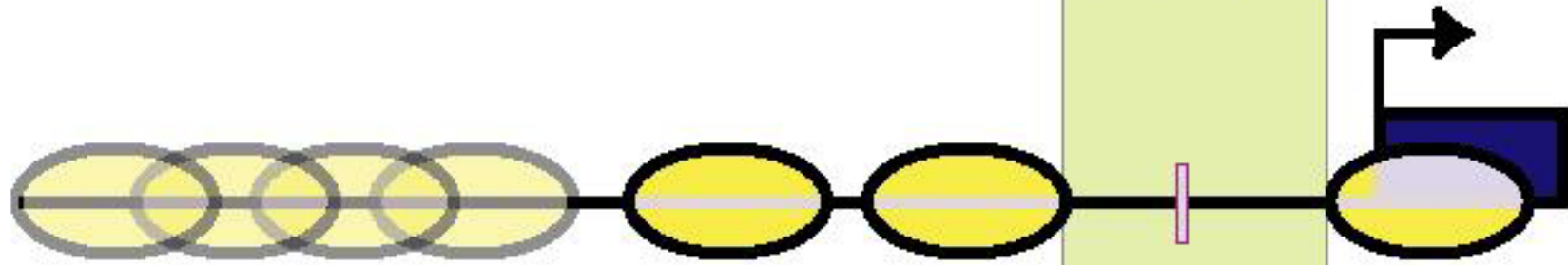
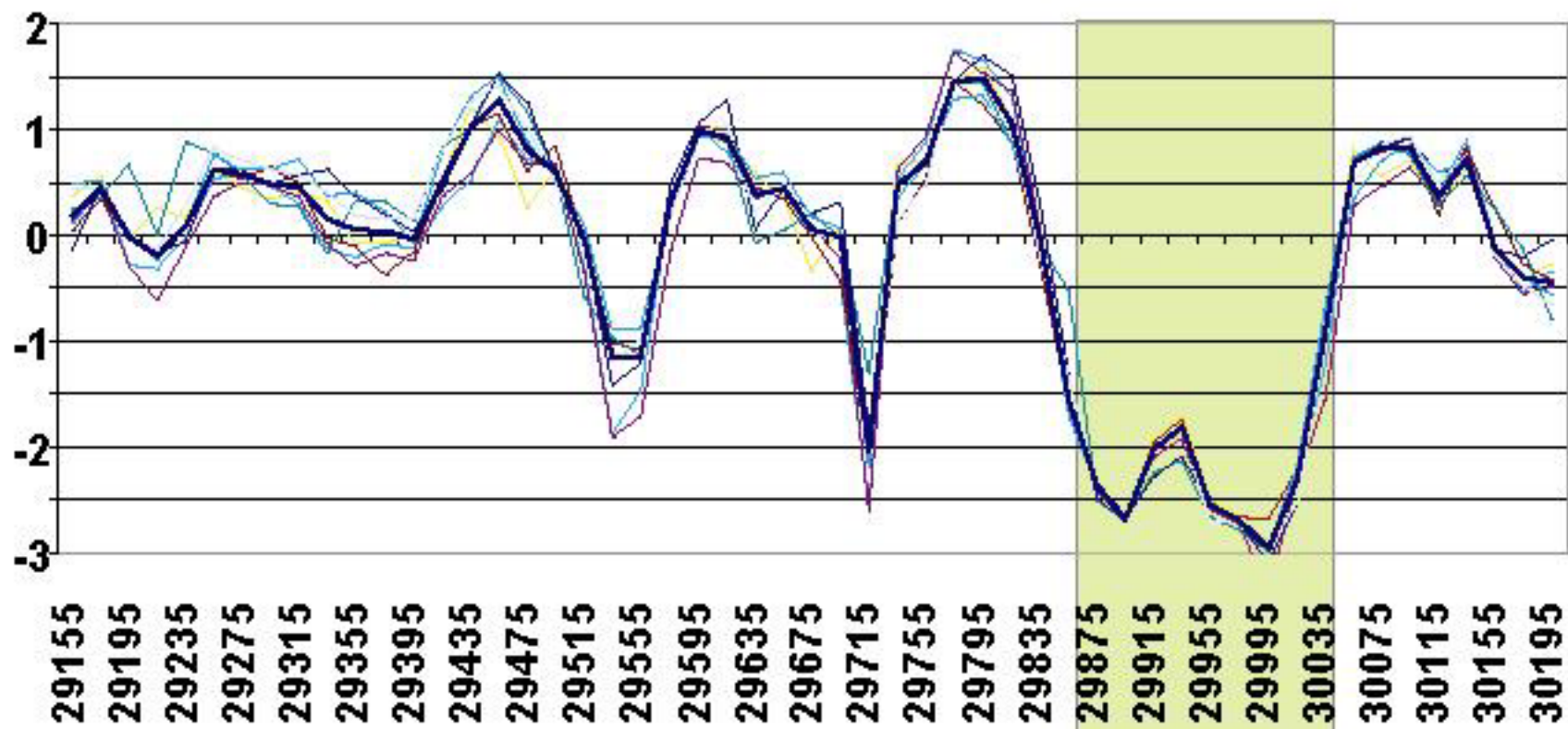
21845 21885 21925 21965 22005 22045 22085 22125 22165 22205 22245 22285 22325 22365 22405 22445 22485 22525 22565 22605 22645 22685 22725 22765 22805 22845 22885 22925

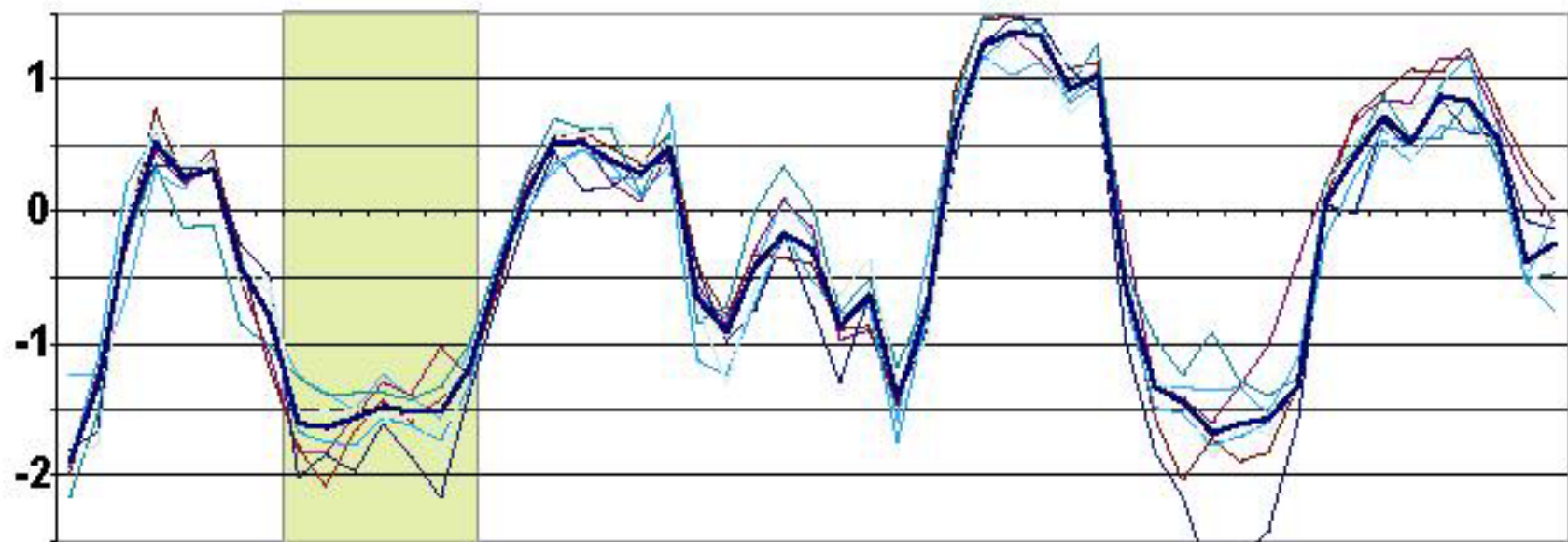




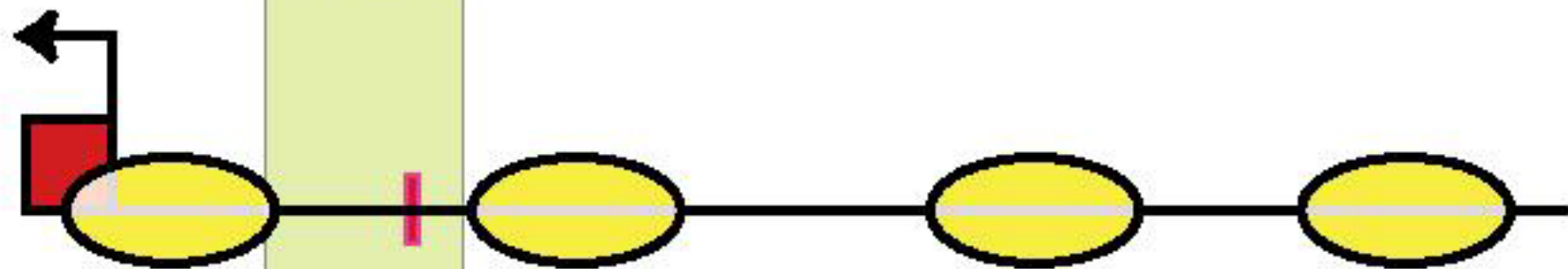
21845 21885 21925 21965 22005 22045 22085 22125 22165 22205 22245 22285 22325 22365 22405 22445 22485 22525 22565 22605 22645 22685 22725 22765 22805 22845 22885 22925







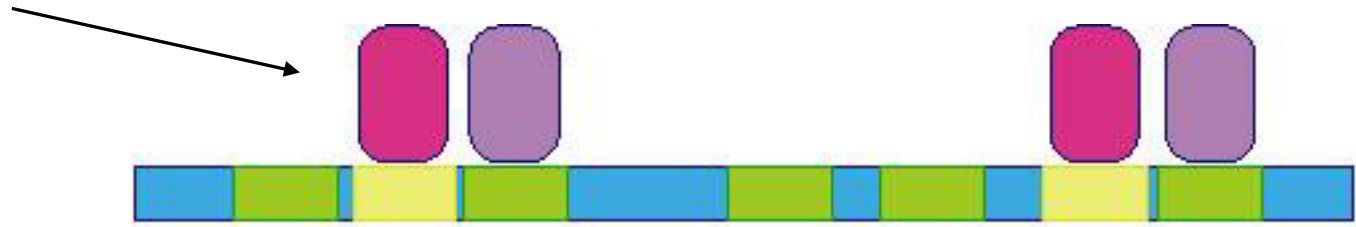
38745 38785 38825 38865 38905 38945 38985 39025 39065 39105 39145 39185 39225 39265 39305 39345 39385 39425 39465 39505 39545 39585 39625 39665 39705 39745 39785



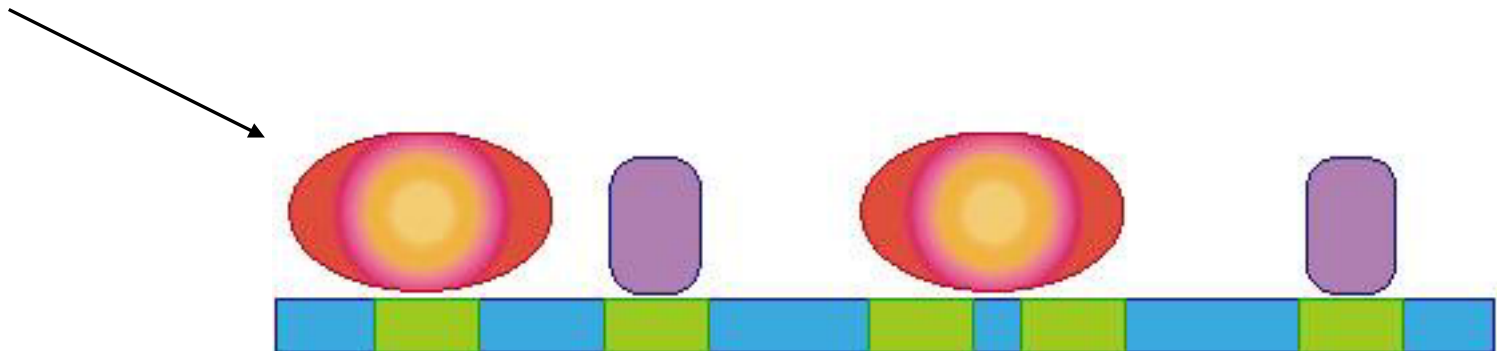
Why do TFs only bind a subset of possible binding sites?



Cooperative binding?

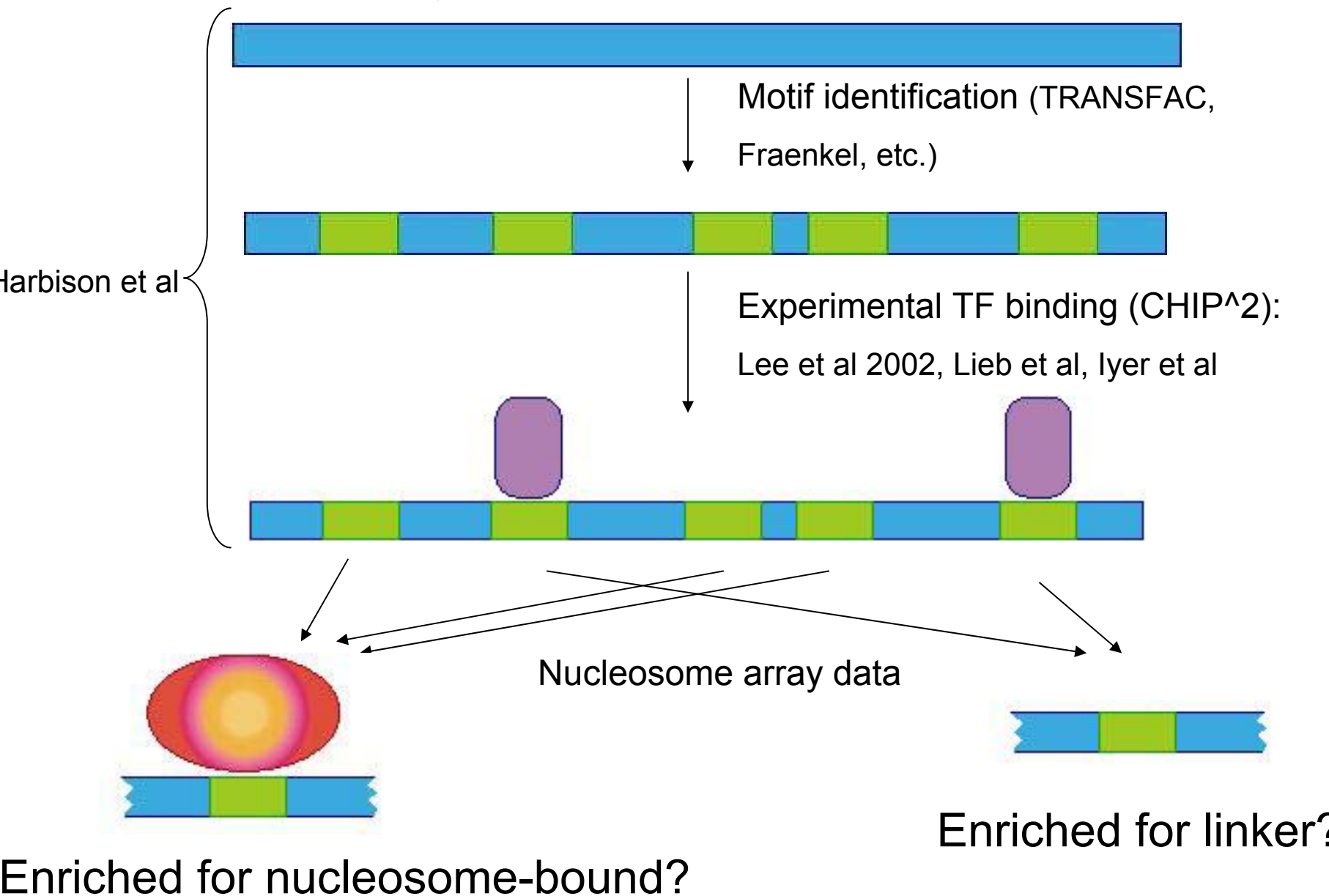


Chromatin?



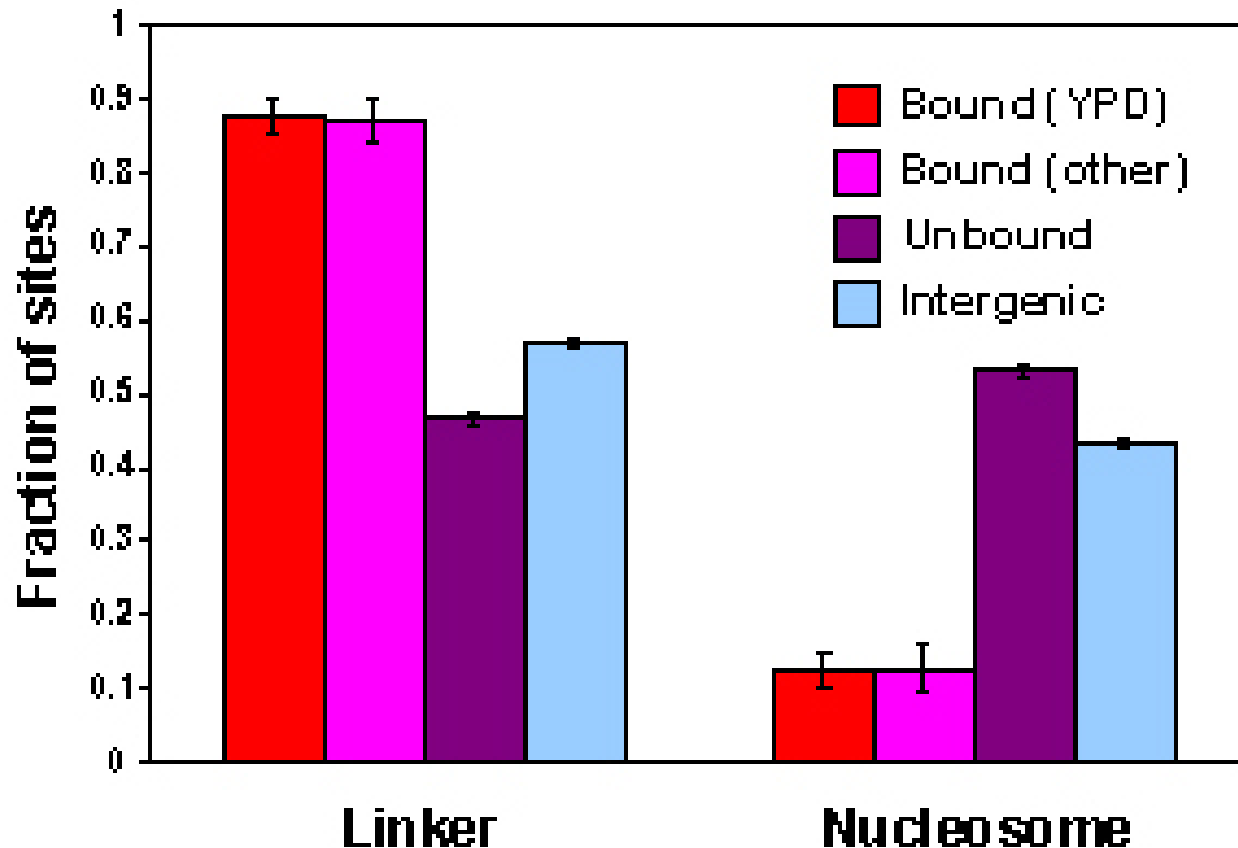
Etc.?

Computational strategy:



Functional TF sites are enriched for linker locations ...

Figure 4



The Histone Code

- There are multiple modifiable amino acids highly conserved in the histone tails

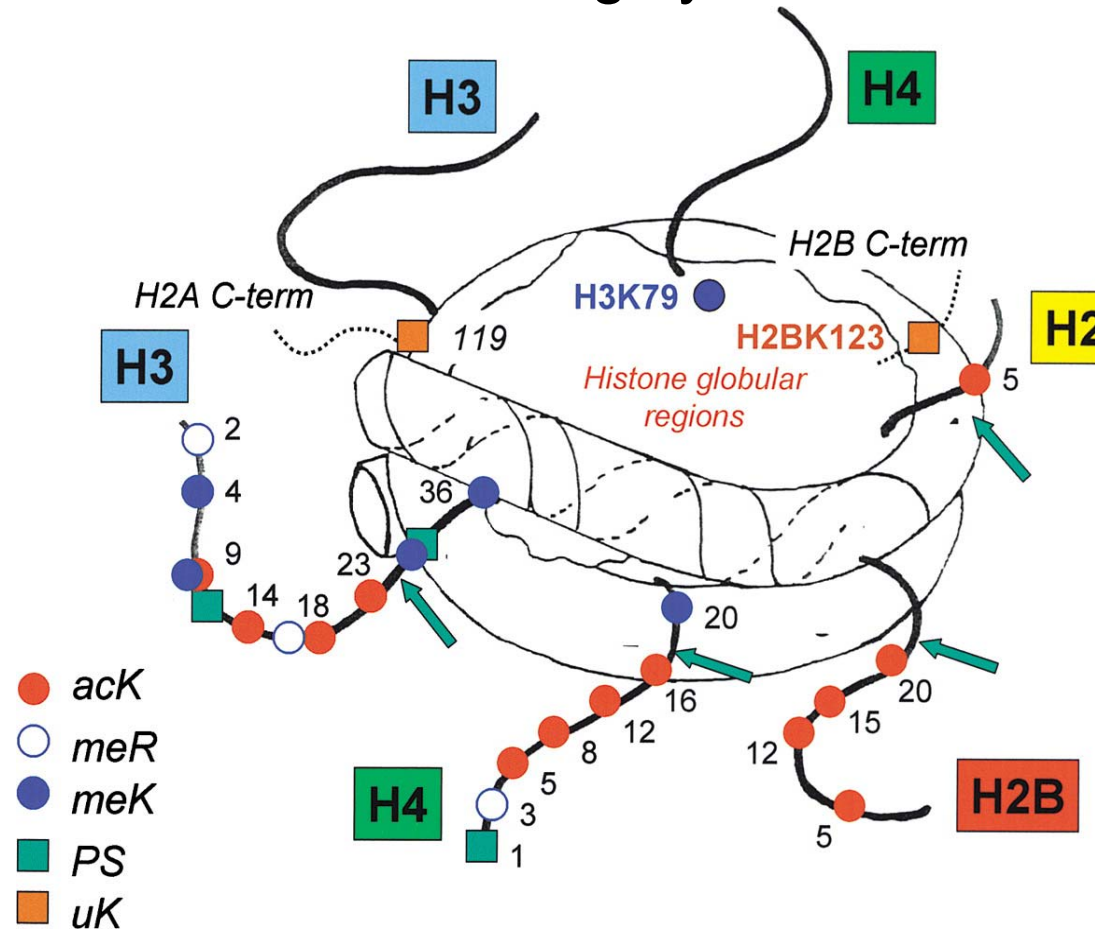
Modifications clearly are involved in regulation of transcription

Code hypothesis – combinatorial use of recruited regulators leads to a rich code specifying transcriptional output

Where are modified nucleosomes positioned in the genome? Maps ...

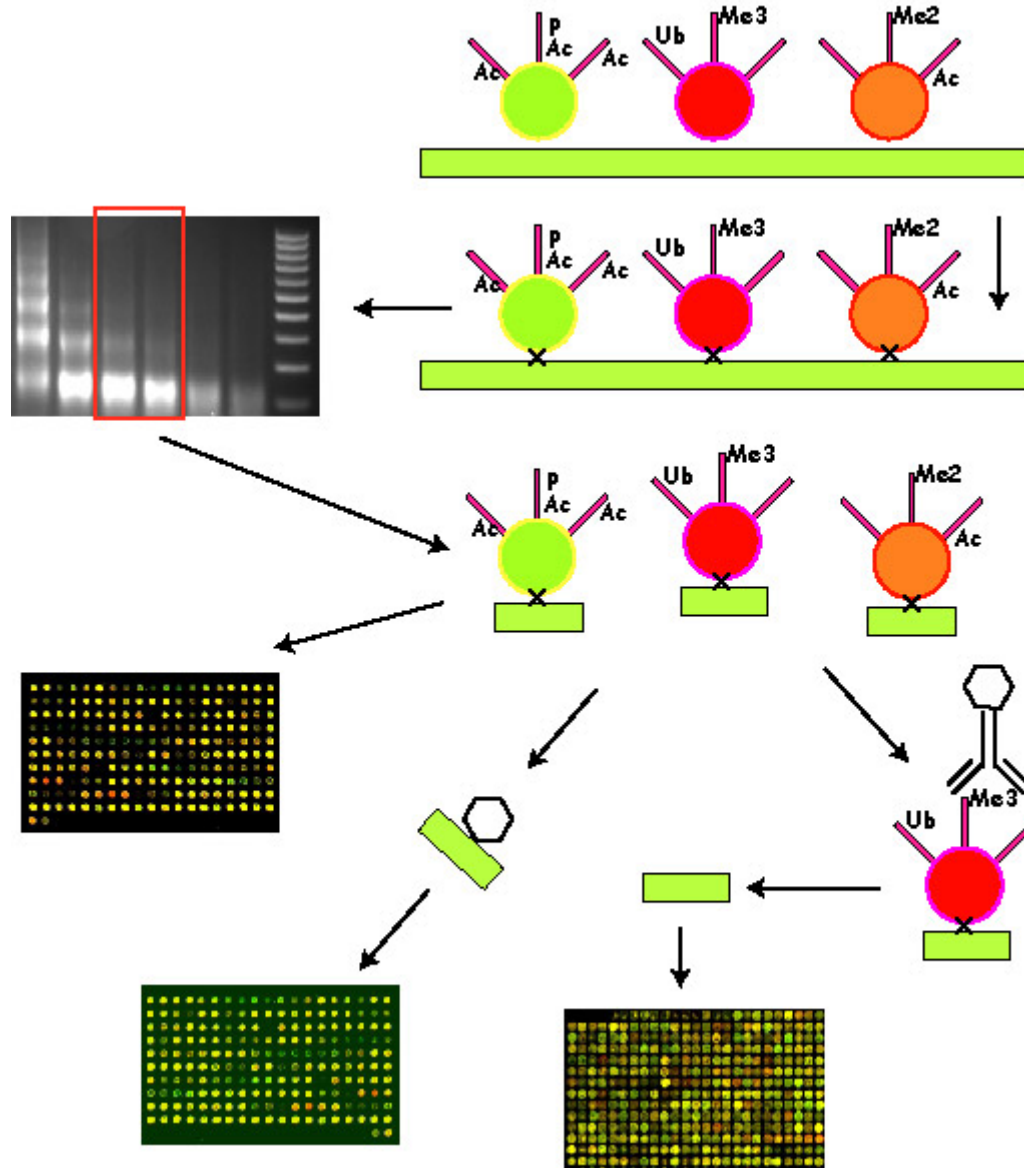
How is the code “read”?

Mod-specific binding vs. charge counters ...

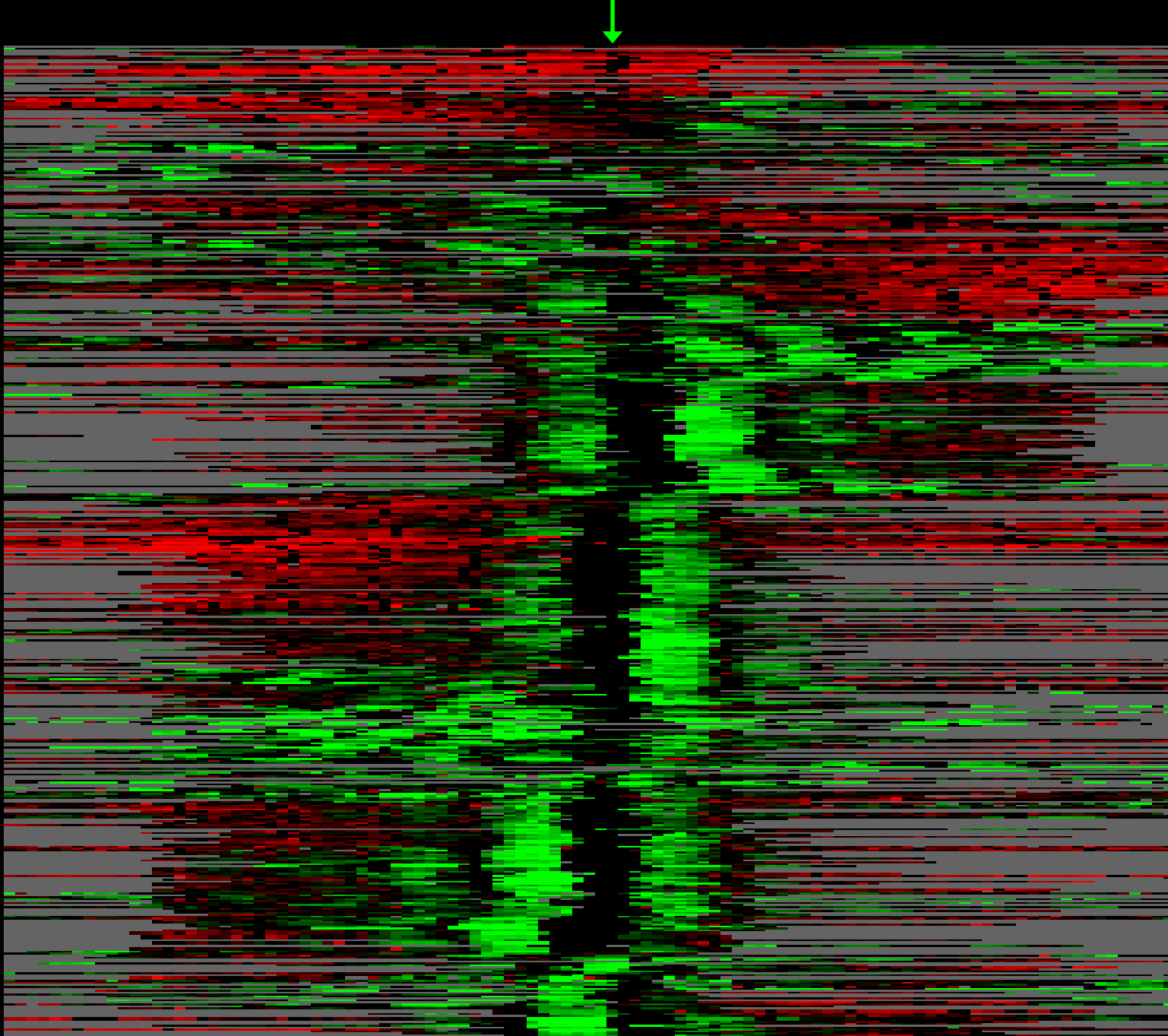


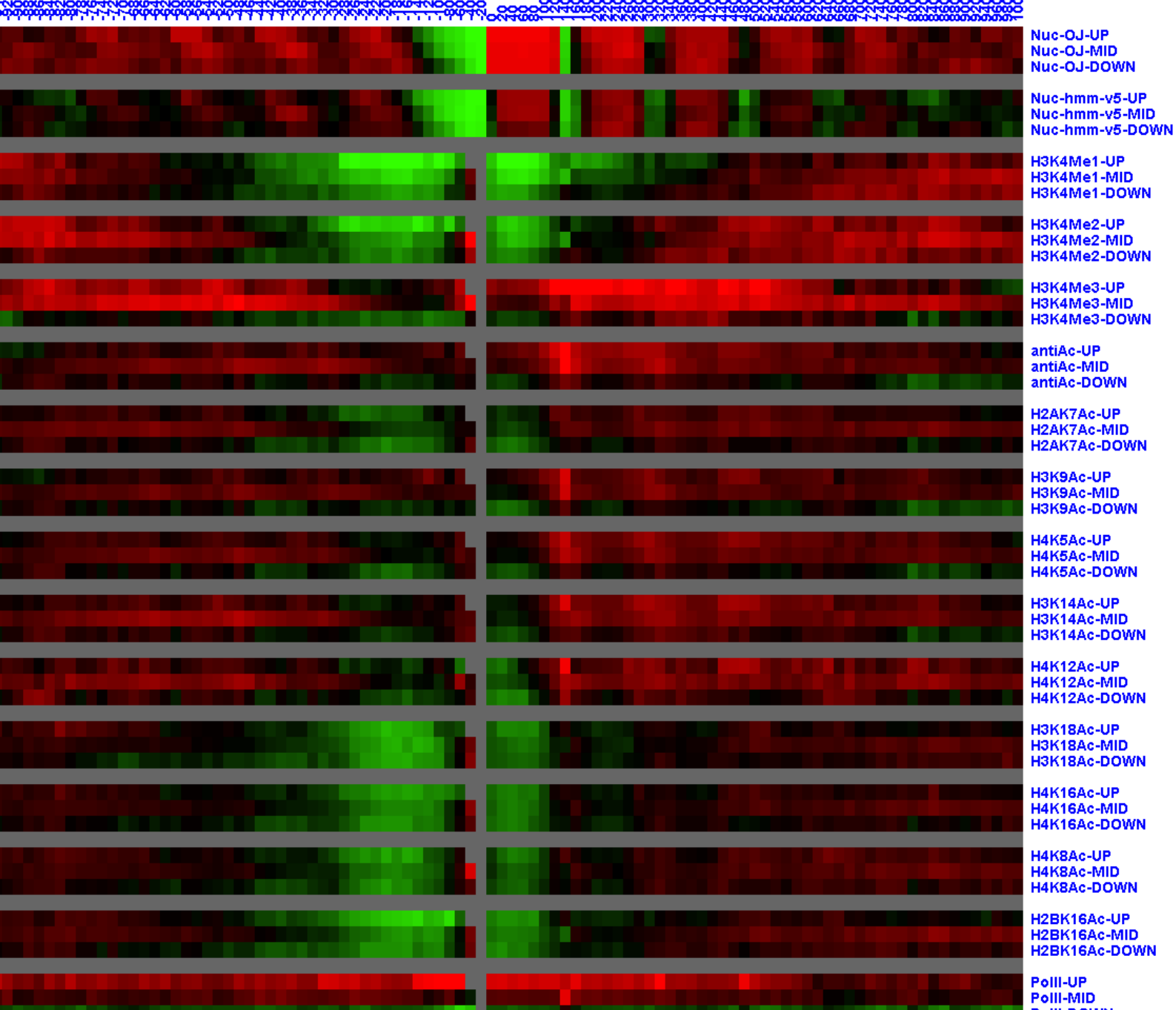
Turner, Cell, 2002

Mapping histone modifications



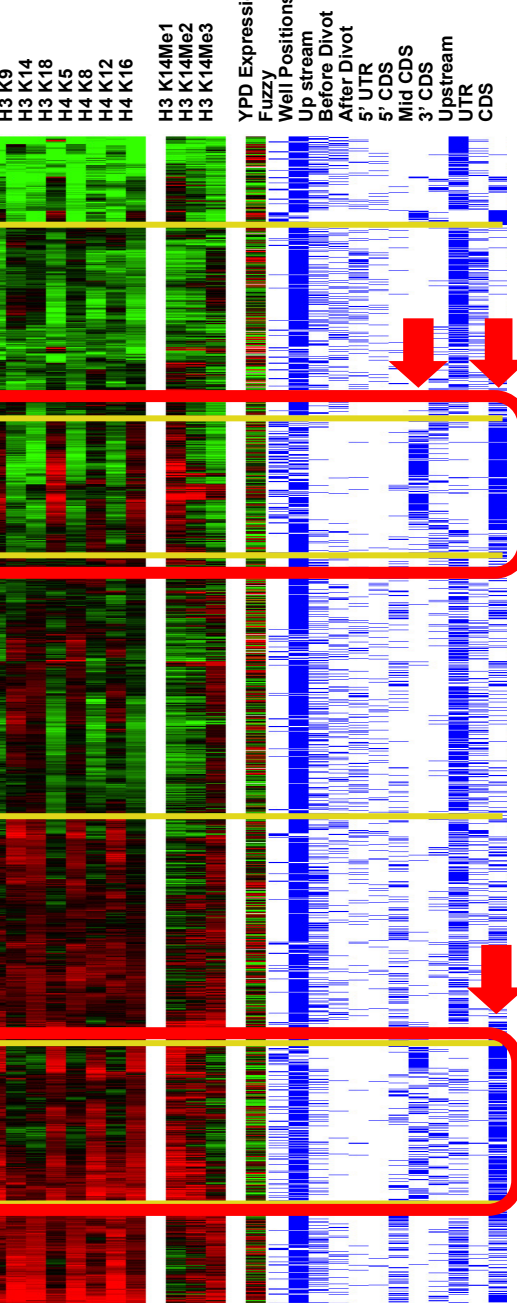
H4 K16
Acetyl,
aligned
by NFR



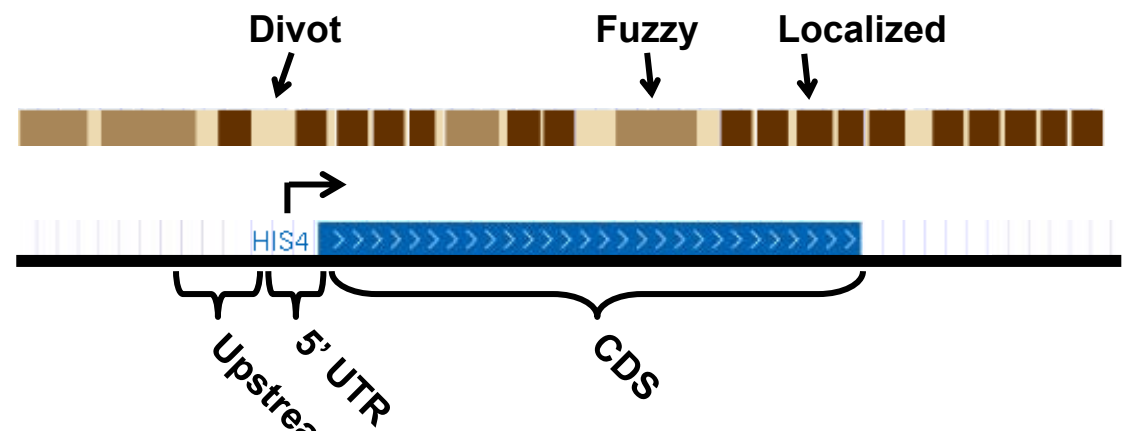
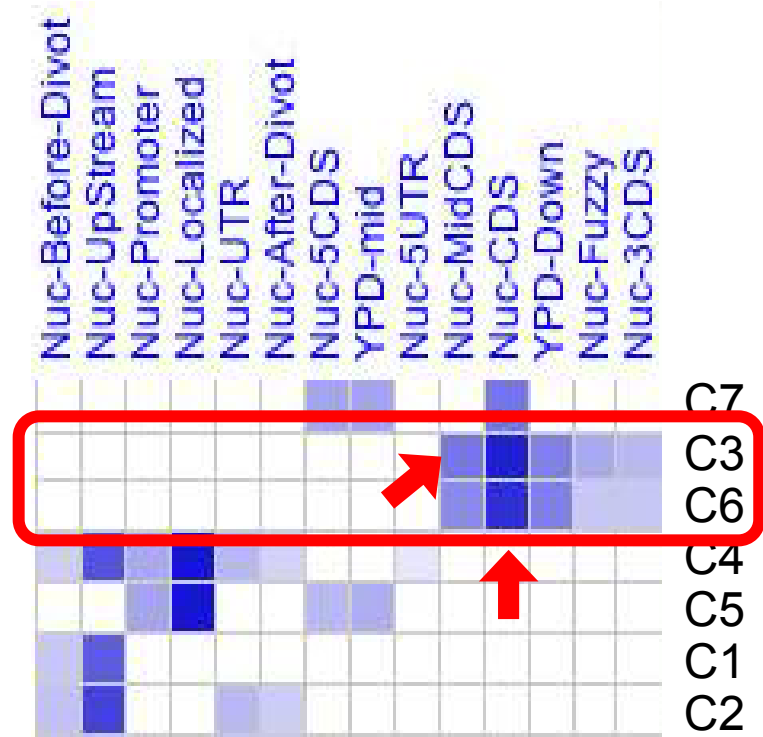


All
mods

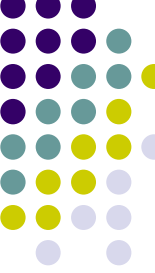
Ac Me Nuc attrs



Nucleosome Flavors



Stereotyped yeast chromatin

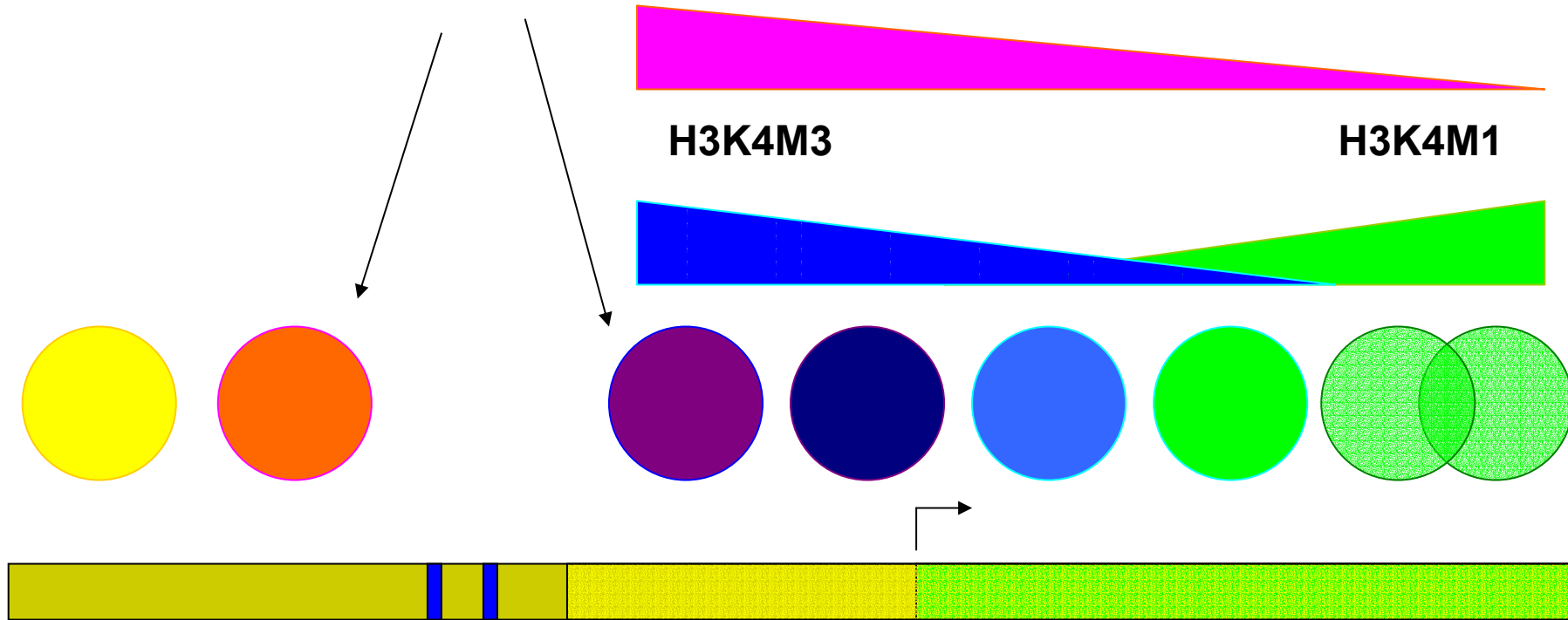


Htz1-containing
nucleosomes, low
H2BK16, H3K18, H4K8,
H4K16 Ac

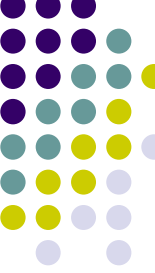
Other acetylation

H3K4M3

H3K4M1



NFR: TF binding. Poly(dA-dT)



Conclusions/questions

- We can “sequence” chromatin in yeast
- What does the sequence tell us?
- Mainly, that yeast promoters all look very much alike. There is not a lot of combinatorial control.
- However, KOs of histone acetylases cause complicated transcriptional changes
- So why does one gene associated with acetylated nucs go up in a HAT KO while another gene goes down?
- What can the primary structure tell us about chromosomal folding?

Acknowledgements



- Steve Buratowski
- Minkyu Kim
- Hiten Madhani
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- Tom Maniatis
- David Haig
- Andrew Murray
- Claire Bailey
- Christian Daley
- Tyler Aldredge
- Everyone else at the CGR!