

PRECISE GENOME-WIDE MAPPING OF SINGLE NUCLEOSOMES AND LINKERS IN VIVO



Răzvan V. Chereji¹, Srinivas Ramachandran², Terri D. Bryson², Steven Henikoff²

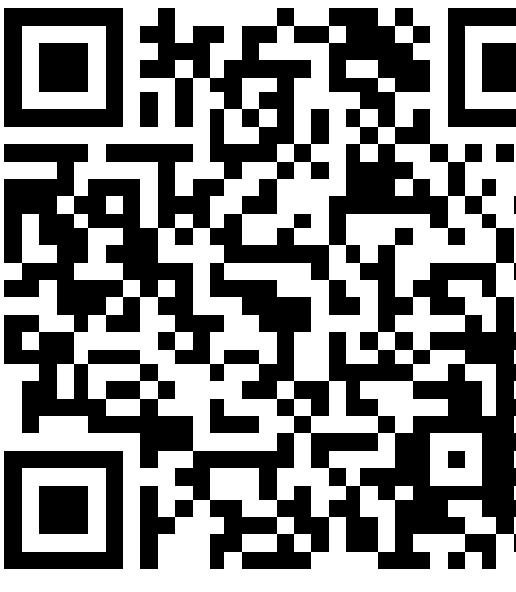
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Eunice Kennedy Shriver
National Institute of
Child Health and
Human Development

¹NICHD, National Institutes of Health, Bethesda, MD 20892, USA

²HHMI and Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

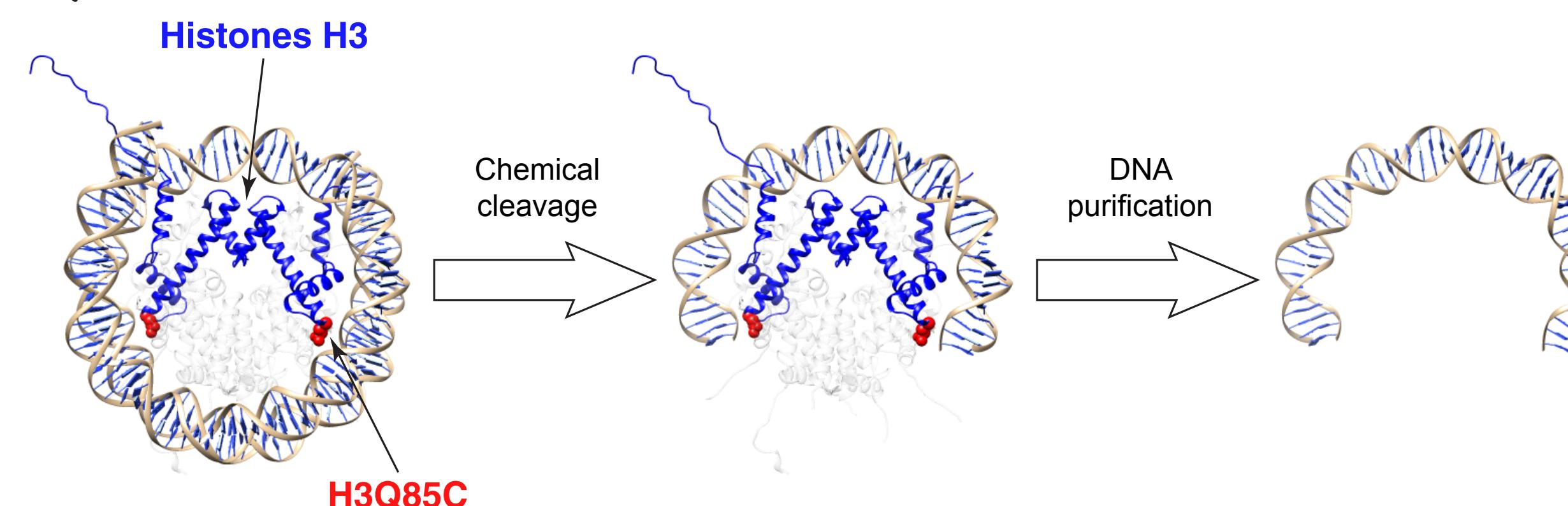


ABSTRACT

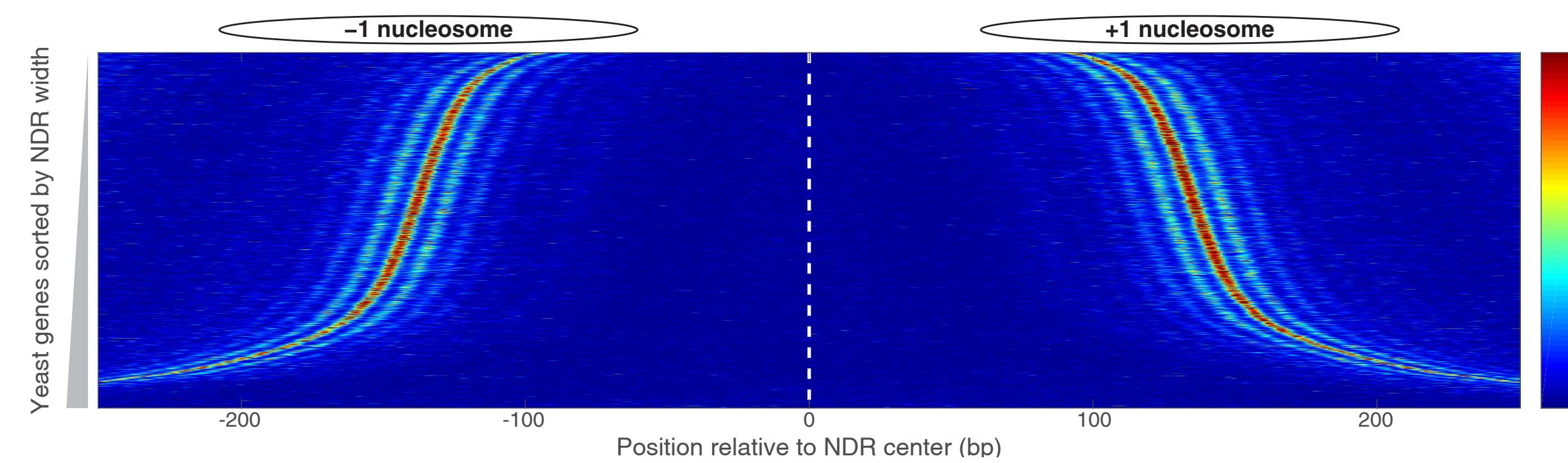
We have developed a chemical cleavage method that allows us to precisely map both single nucleosomes and linkers in budding yeast. Its high resolution allows us to distinguish alternative rotational positions that nucleosomes occupy in different cells and to demonstrate that linker DNA has “quantized” lengths in individual genes. Furthermore, we evaluate the contributions of sequence, transcription, histone H1 and the H2A.Z variant in defining the chromatin landscape. We show that DNA sequence has a limited effect on establishing the nucleosome organization as observed in vivo. Moreover, we find that the degree of gene compaction, measured by the spacing between neighboring nucleosomes, correlates with the transcription level, amount of histone H1 bound to the gene, and the amount of H2A.Z variant that is incorporated in the +1 nucleosomes. Furthermore, we present a biophysical model, which shows that steric exclusion between neighboring nucleosomes suffices to explain the complex nucleosome phasing pattern that is observed near the gene ends.

MAPPING OF NUCLEOSOMES

H3Q85C cleavages produce 50 bp DNA fragments that are symmetrical relative to the nucleosome dyads.

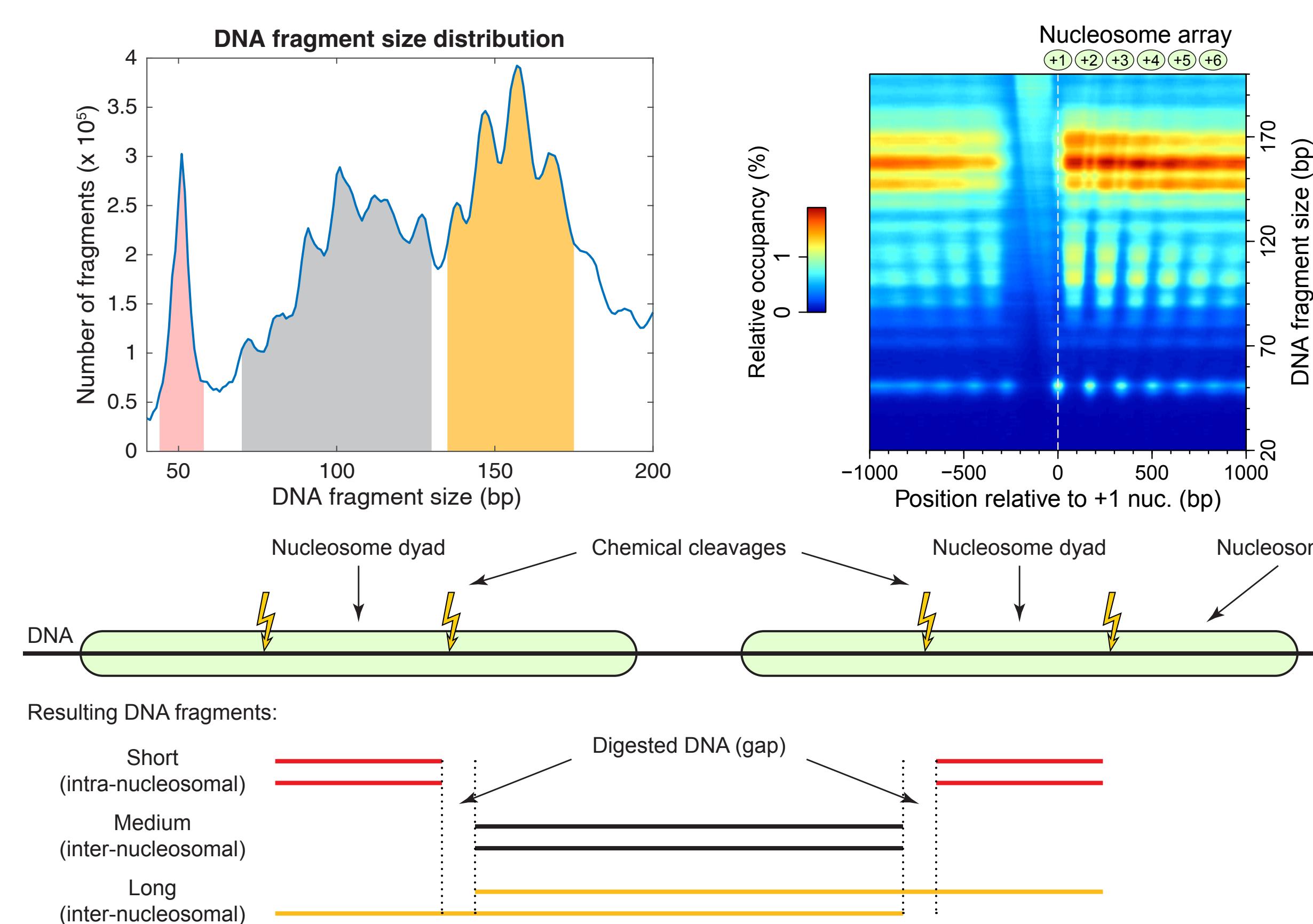


The center of each fragment indicates the position of a nucleosome dyad with very high accuracy.

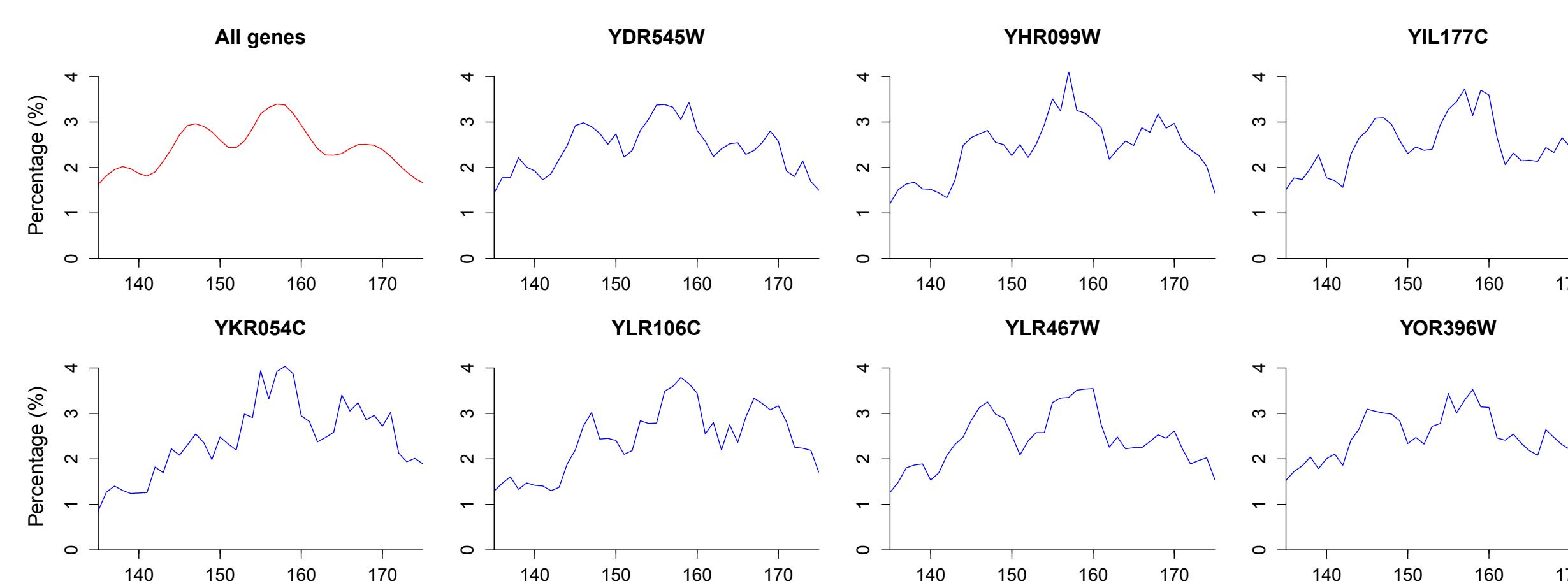


LINKER “QUANTIZATION”

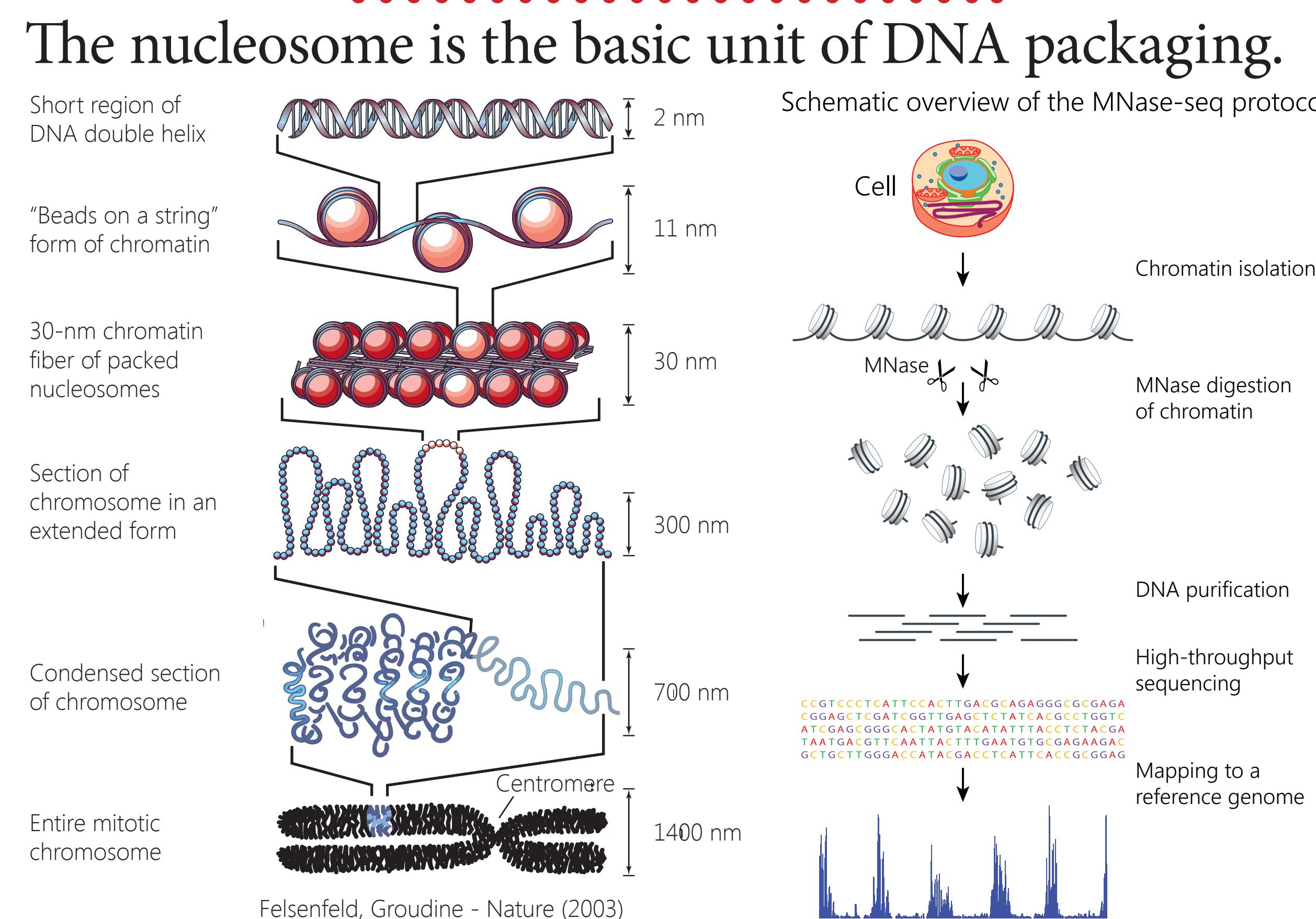
We can measure the spacing between neighboring nucleosomes from the same cell.



We obtain the preferred spacing: 151, 161, 171 bp. Preferred linker lengths: 5, 15, 25 bp or 4, 14, 24 bp – depending on the length of nucleosomal DNA (146 bp or 147 bp, respectively). This linker “quantization” rule is true even for single genes.

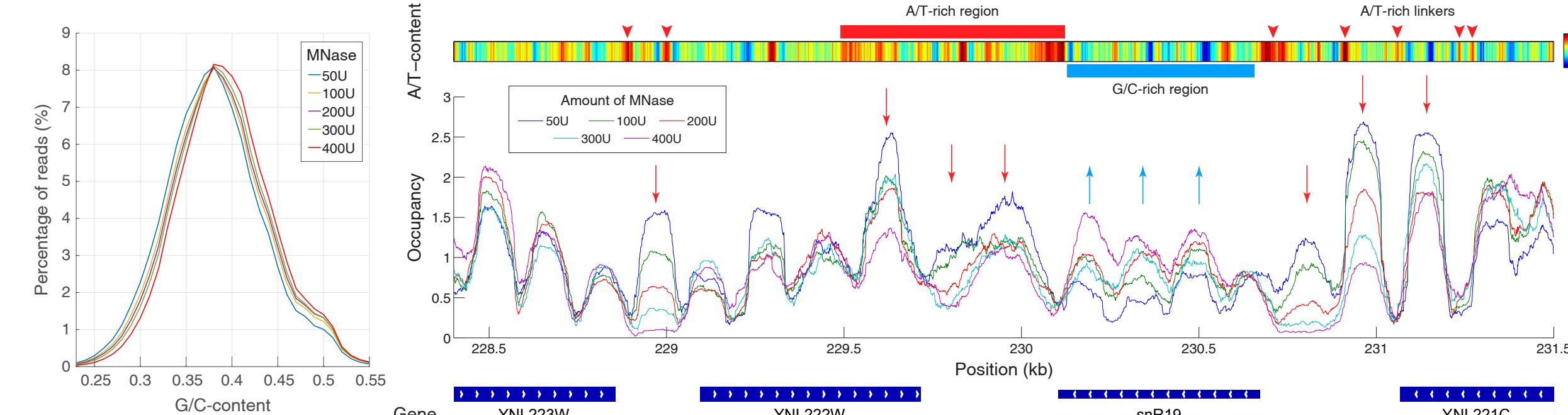


DNA PACKAGING



MNASE SENSITIVITY

MNase has a strong DNA sequence specificity [1,2].

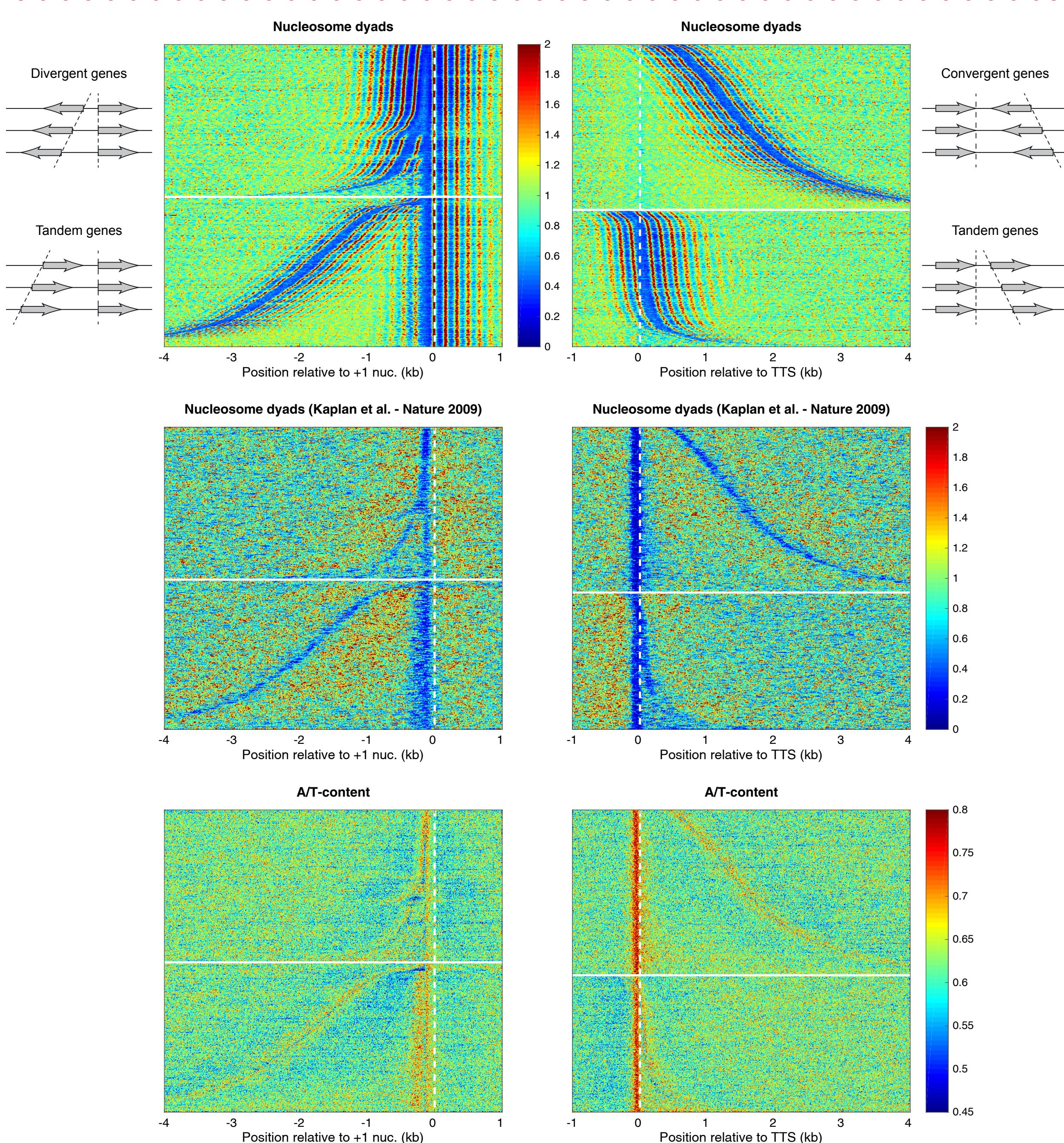


To eliminate the bias introduced by MNase, we developed a new method of mapping nucleosomes and linker positions genome-wide [3].

CORRELATIONS BETWEEN NUC. SPACING AND OTHER DATA

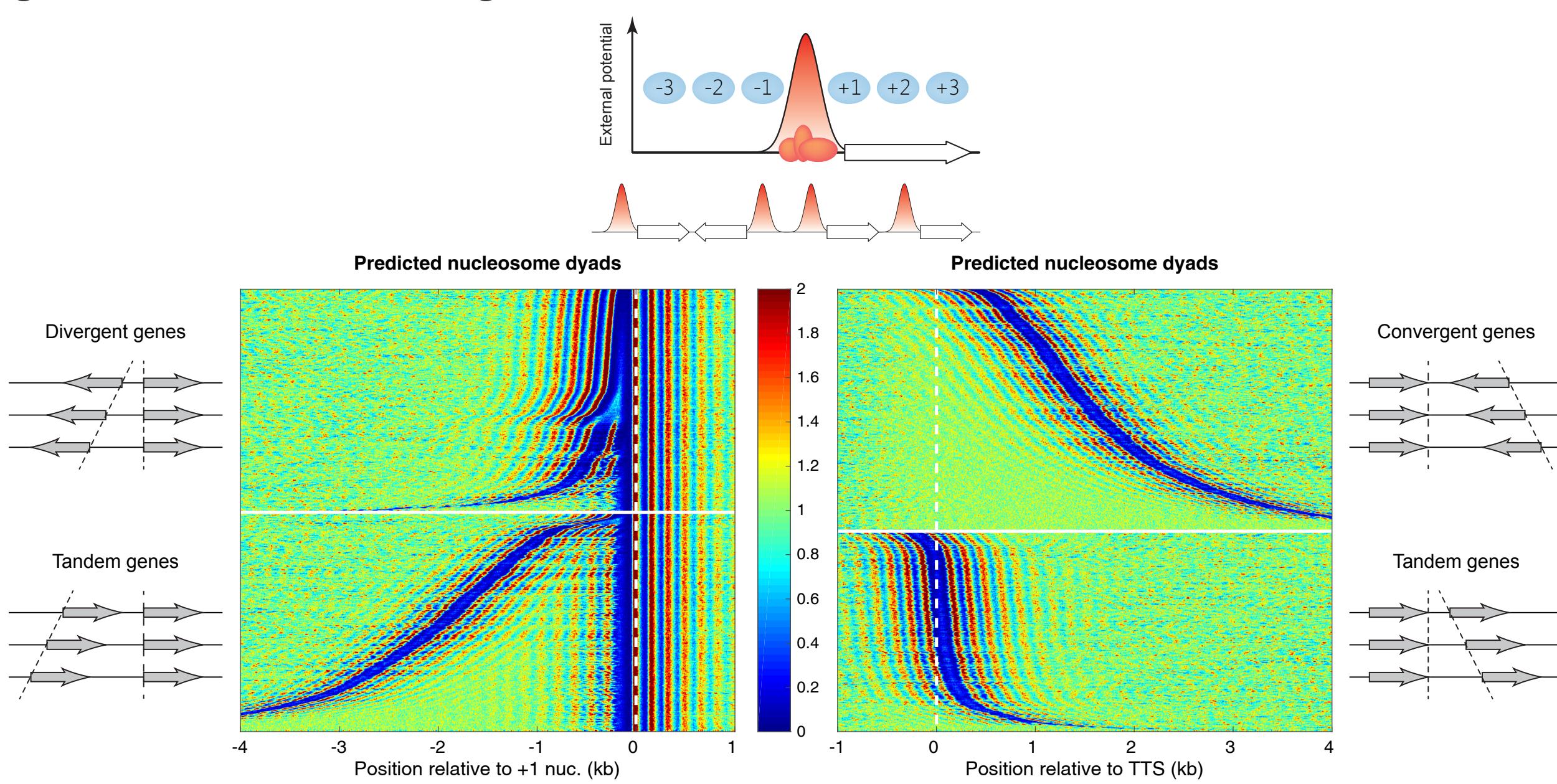
Quintile	Spacing	NDR width	NET-seq	Rpb1	Rpb3	H1	H2A.Z	H3K4me3	TBP
1	160.4	144	1.020	1.750	1.419	0.919	2.856	2.365	3.305
2	164.8	134	0.908	1.402	1.212	0.989	3.299	2.110	2.985
3	166.8	130	0.846	1.225	1.104	1.030	3.565	1.903	2.694
4	167.5	125	0.694	0.843	0.883	1.099	3.787	1.693	2.467
5	169.8	120	0.636	0.741	0.804	1.210	4.053	1.458	2.361

NUCLEOSOME POSITIONING “CODE”



BIOPHYSICAL MODEL

Using statistical mechanics [3,4,5,6] we predict the genome-wide organization of nucleosomes.



REFERENCES

- [1] RV Chereji*, J Ocampo*, DJ Clark, Mol. Cell 65, 565 (2017) (*co-first authors)
- [2] RV Chereji*, T-W Kan* et al., Nucleic Acids Res. 44, 1036 (2016) (*co-first authors)
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- [4] RV Chereji et al., Phys. Rev. E 83, 050903 (2011)
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