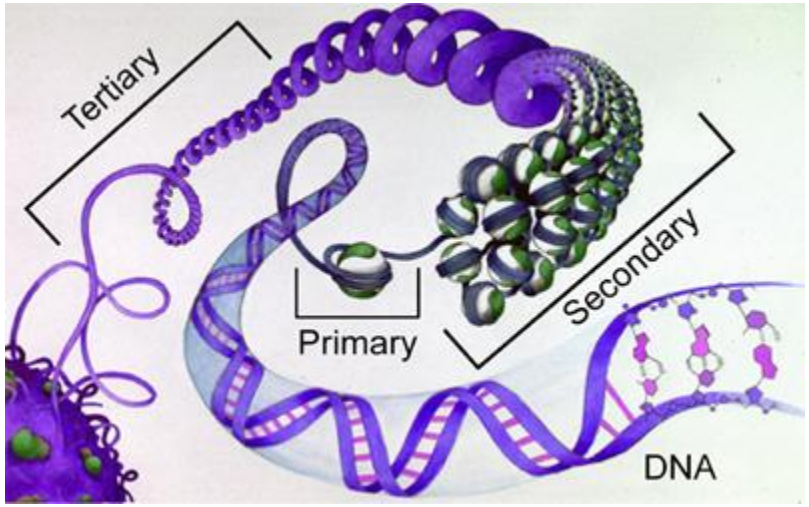
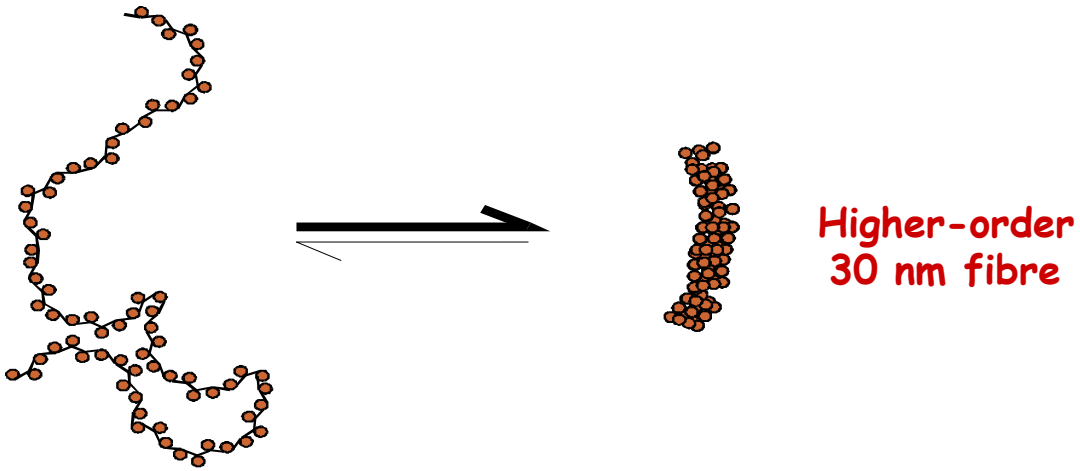
DNA sequence and chromatin structure



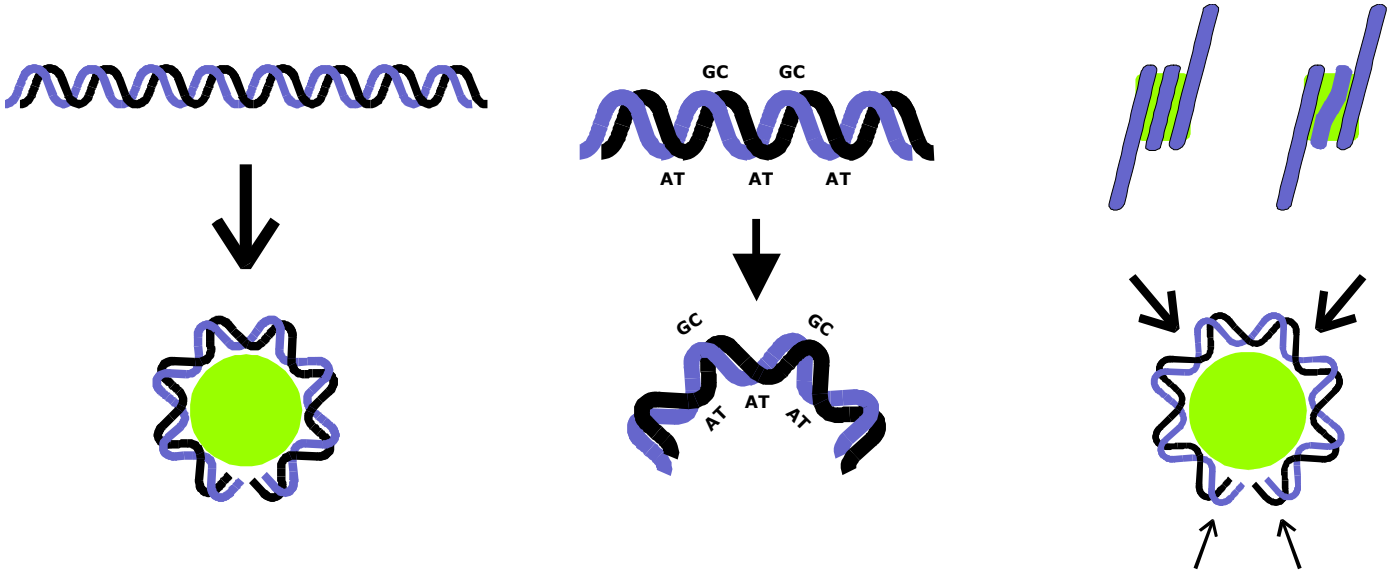
Mapping nucleosome positioning using high-throughput sequencing

DNA sequence and chromatin structure



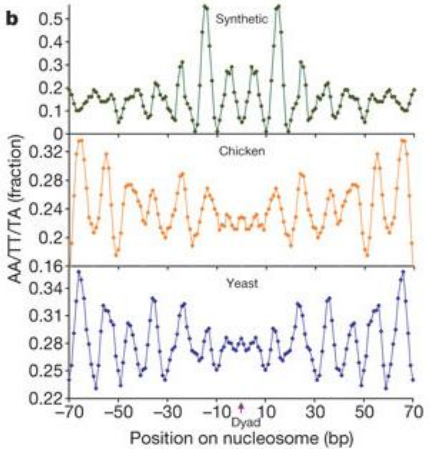
Mapping nucleosome positioning using high-throughput sequencing

DNA sequence-directed nucleosome positioning



DNA is tightly bent onto the nucleosome surface
Sequence distribution facilitates DNA bending
Nucleosomal DNA is not smoothly bent

Chromatin Code Decoded?



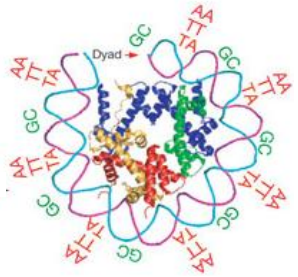
nature Vol 442|17 August 2006|doi:10.1038/nature04979

ARTICLES

A genomic code for nucleosome positioning

Eran Segal[1], Yvonne Fondufe-Mittendorf[2], Lingyi Chen[2], AnnChristine Thåström[2], Yair Field[1], Irene K. Moore[2], Ji-Ping Z. Wang[3] & Jonathan Widom[2]

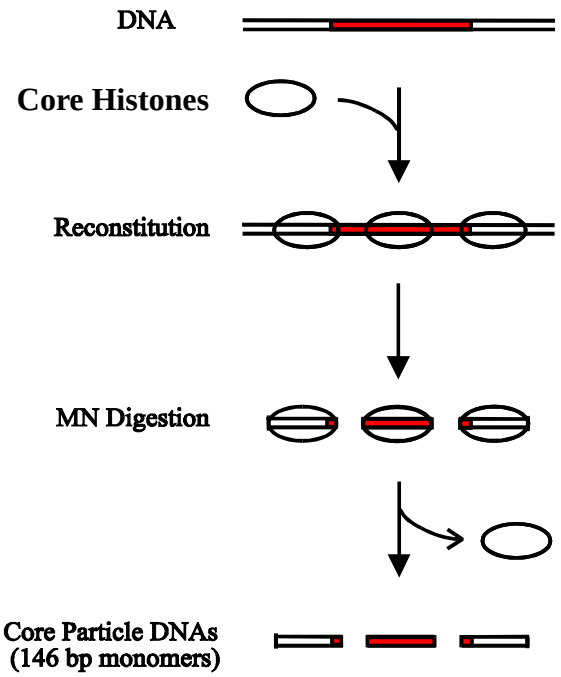
Eukaryotic genomes are packaged into nucleosome particles that occlude the DNA from interacting with most DNA binding proteins. Nucleosomes have higher affinity for particular DNA sequences, reflecting the ability of the sequence to bend sharply, as required by the nucleosome structure. However, it is not known whether these sequence preferences have a significant influence on nucleosome position *in vivo*, and thus regulate the access of other proteins to DNA. Here we isolated nucleosome-bound sequences at high resolution from yeast and used these sequences in a new computational approach to construct and validate experimentally a nucleosome–DNA interaction model, and to predict the genome-wide organization of nucleosomes. Our results demonstrate that genomes encode an intrinsic nucleosome organization and that this intrinsic organization can explain ~50% of the *in vivo* nucleosome positions. This nucleosome positioning code may facilitate specific chromosome functions including transcription factor binding, transcription initiation, and even remodelling of the nucleosomes themselves.

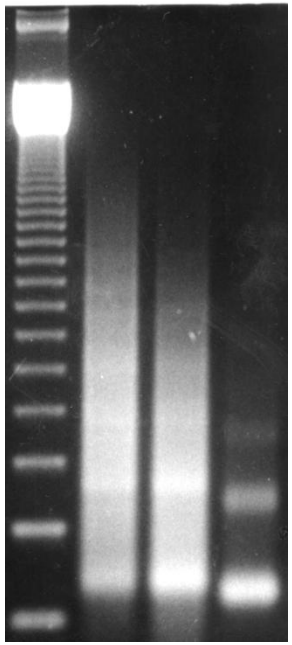


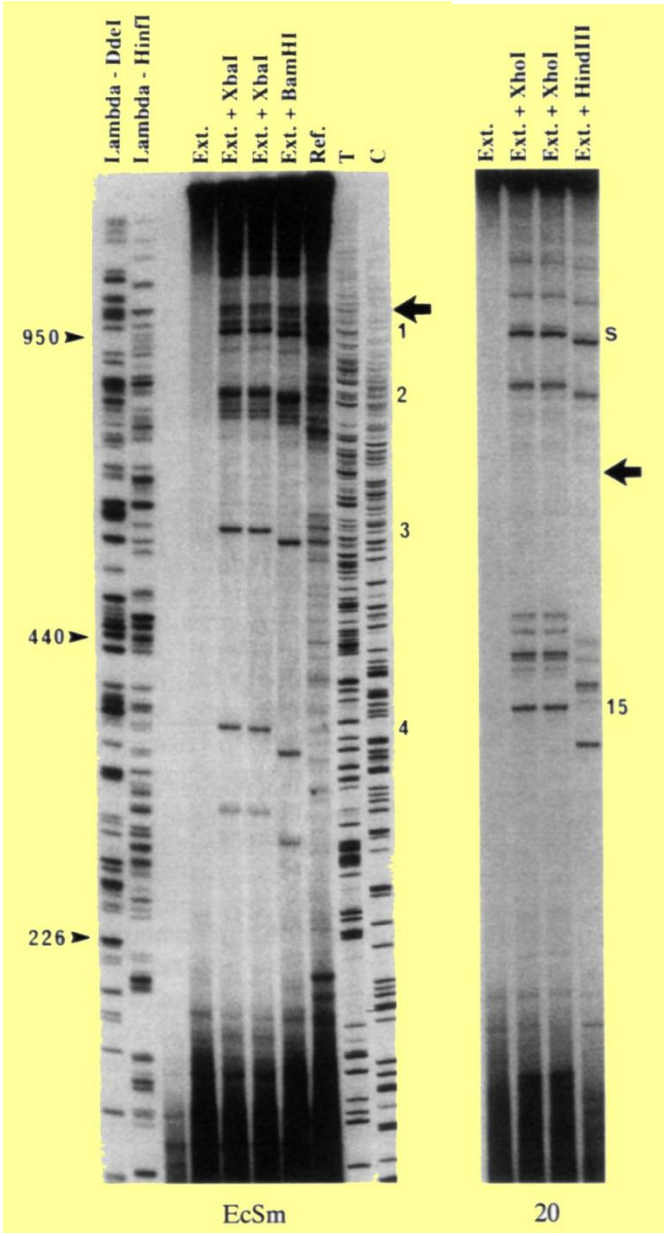
90% of *in vivo* nucleosome positioning in yeast, is directed by DNA sequence.

Mapping nucleosome positioning sites by monomer extension

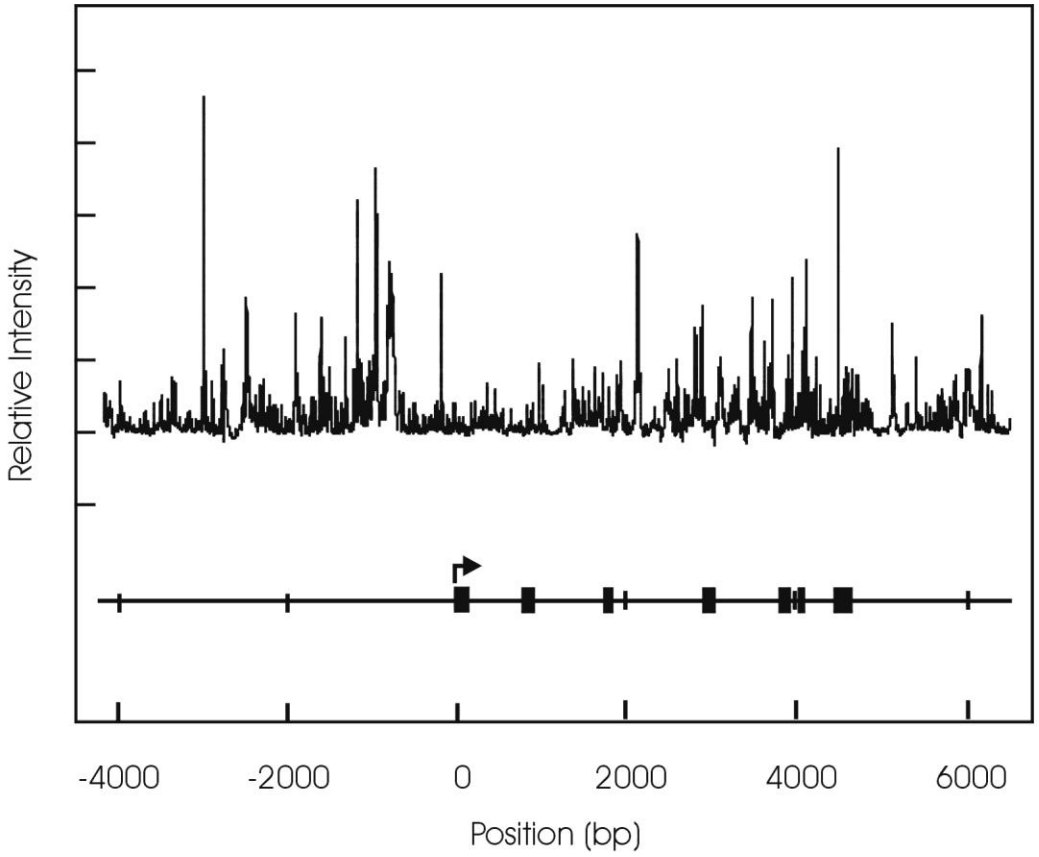
Preparation of core particle DNA population

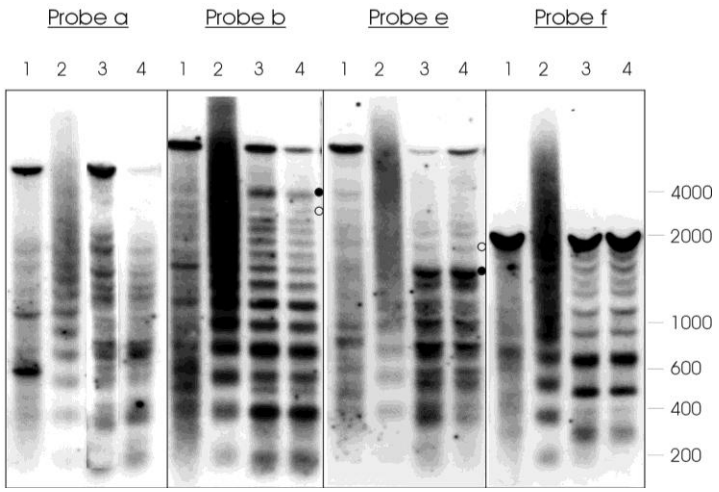


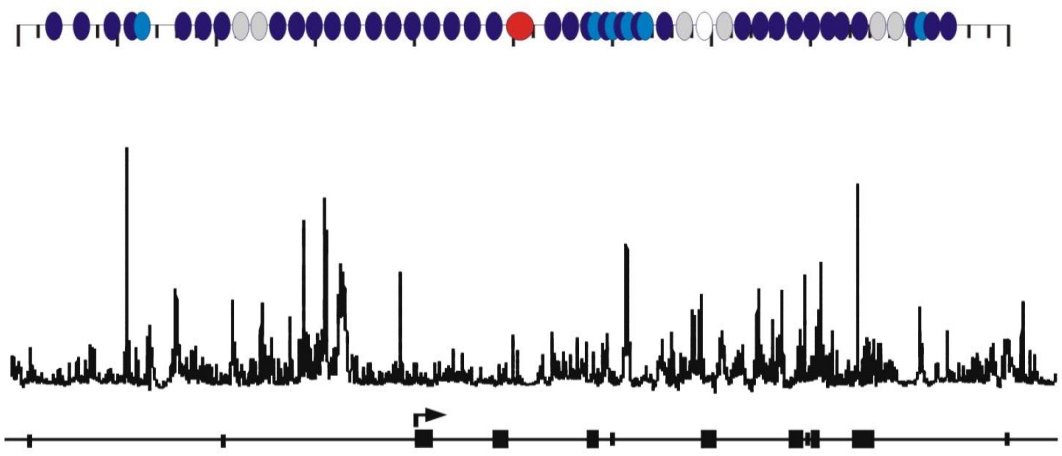




In vitro nucleosome map for the β-lactoglobulin gene (BLG)







Nucleosome positioning *in vivo*

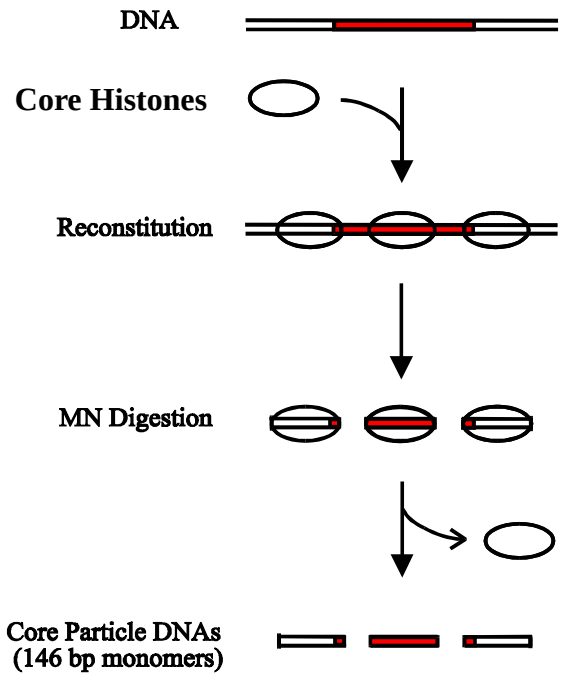
(Sheep liver)

***In vivo* and *in vitro* nucleosome positioning are related.**

Gencheva et al. (2006)
J. Mol. Biol. 361, 216-230

Mapping nucleosome positioning sites by high-throughput sequencing

Preparation of core particle DNA population



Mapping Nucleosome positioning sites

(Roche 454)

BLG DNA (12,861 bp)

152,000 reads
Average read length ~ 145 bp

Coverage (per nucleotide)

1700 fold

Yeast ~ 1.5
Human ~ 0.001

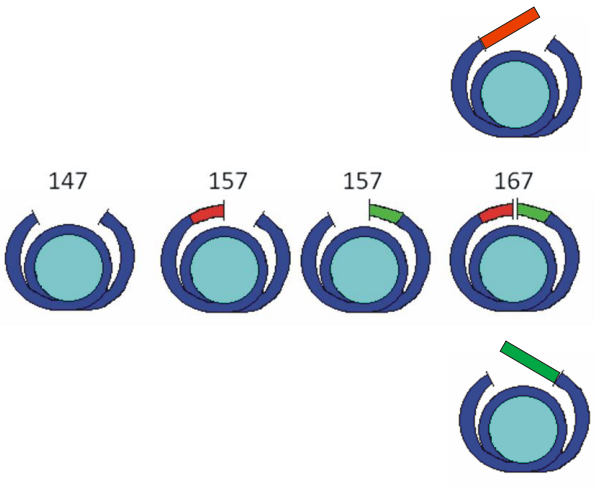
Mapping Nucleosome positioning sites

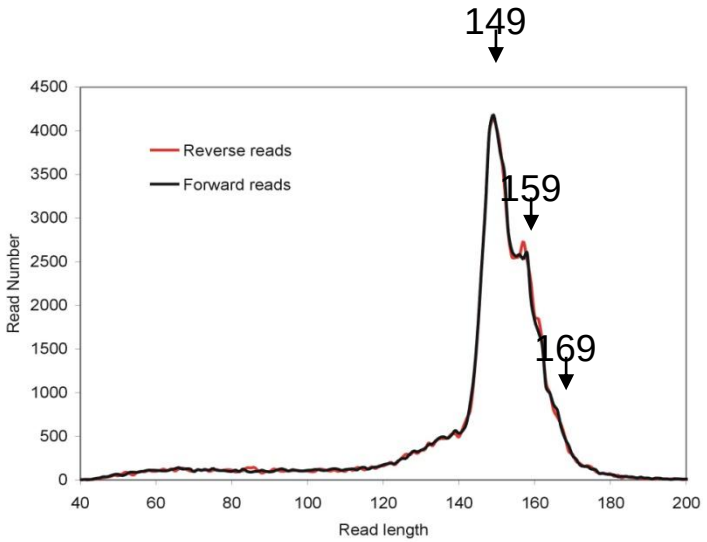
1. Assume a fixed nucleosome size (149 bp) and realign forward and reverse reads to identify position and amplitude nucleosome centre (dyad).

2. Accommodate nucleosomal DNA size variation by using a variable window to match forward and reverse reads to identify position and amplitude of dyad.

3. Use only particular window (nucleosome) sizes (149, 159, 169) to identify position and amplitude of dyad.

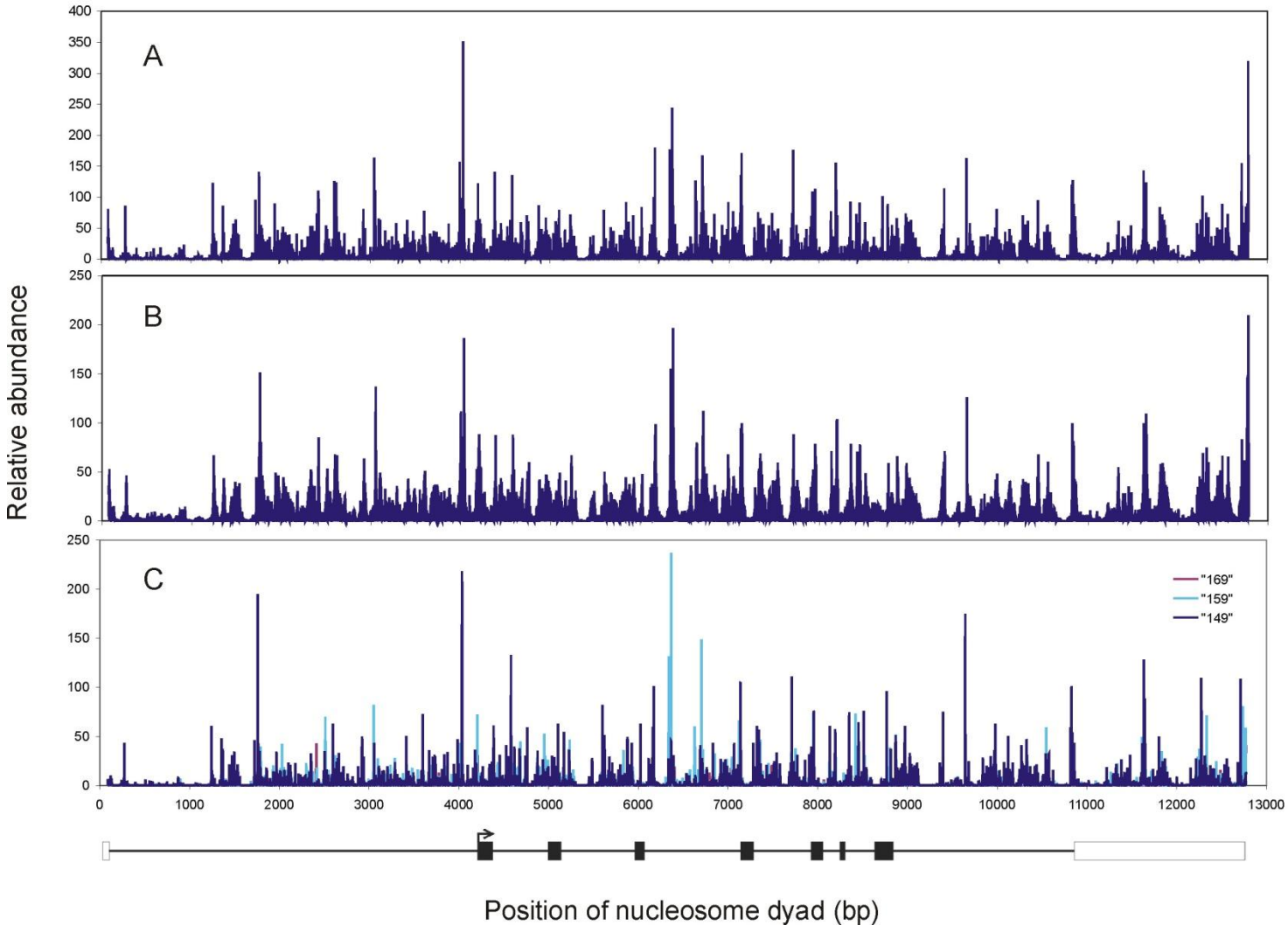
Discrete Nucleosome Sizes





The histone octamer packages/protects DNA in units of 10 bp and this packaging can extend beyond the limits of the core particle

Histone octamer positioning maps

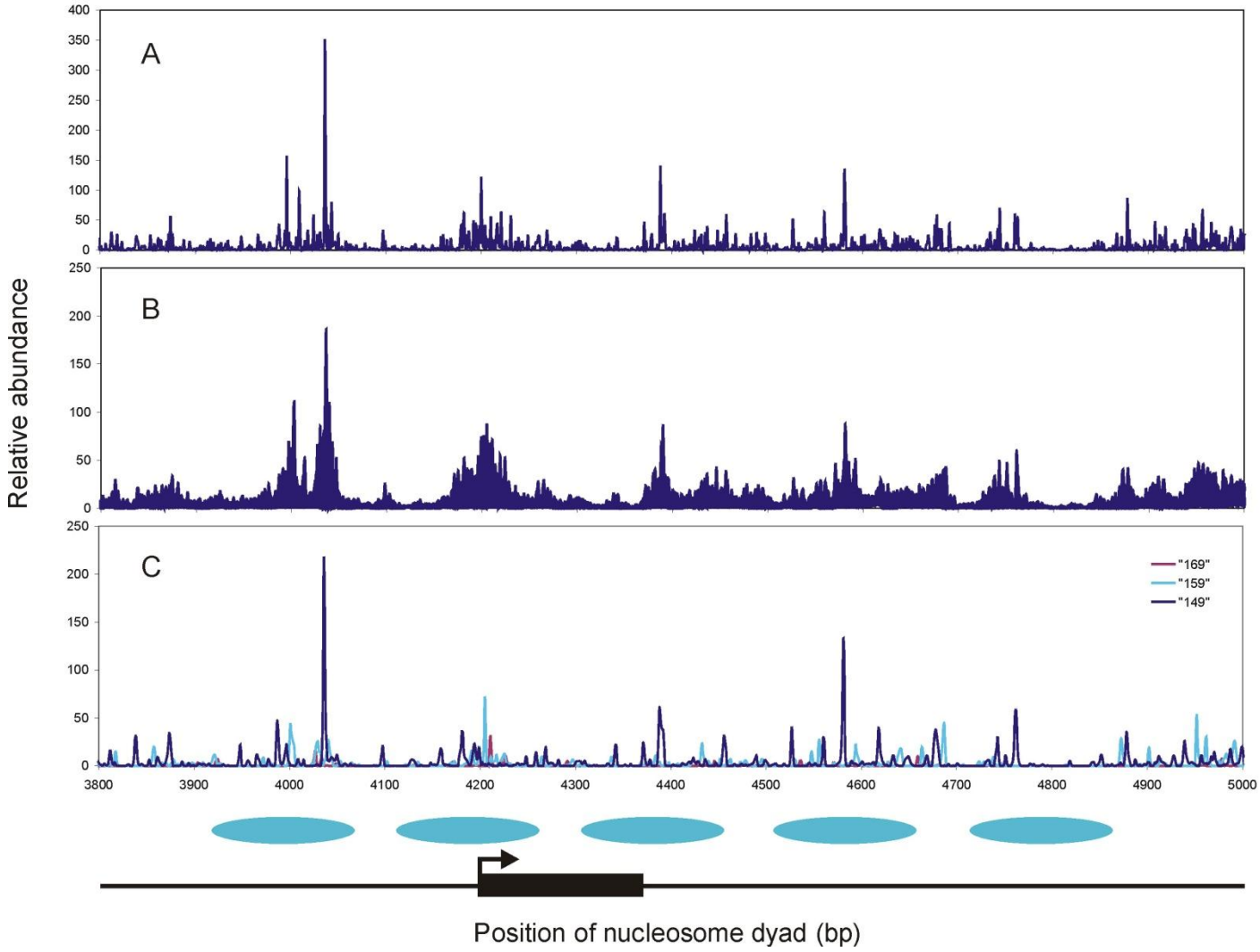


Method 1
1 map
(149 bp)

Method 2
16 maps
(141, 143…171 bp)

Method 3
3 maps
(149, 159, 169 bp)

Histone octamer positioning maps: The BLG promoter region



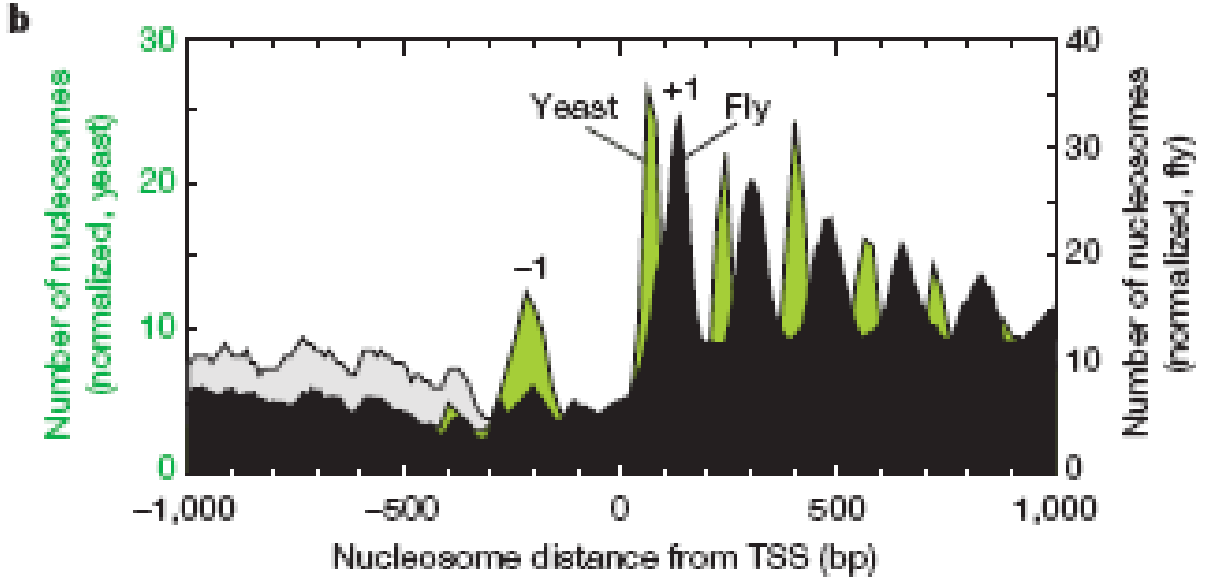
Method 1

Method 2

Method 3

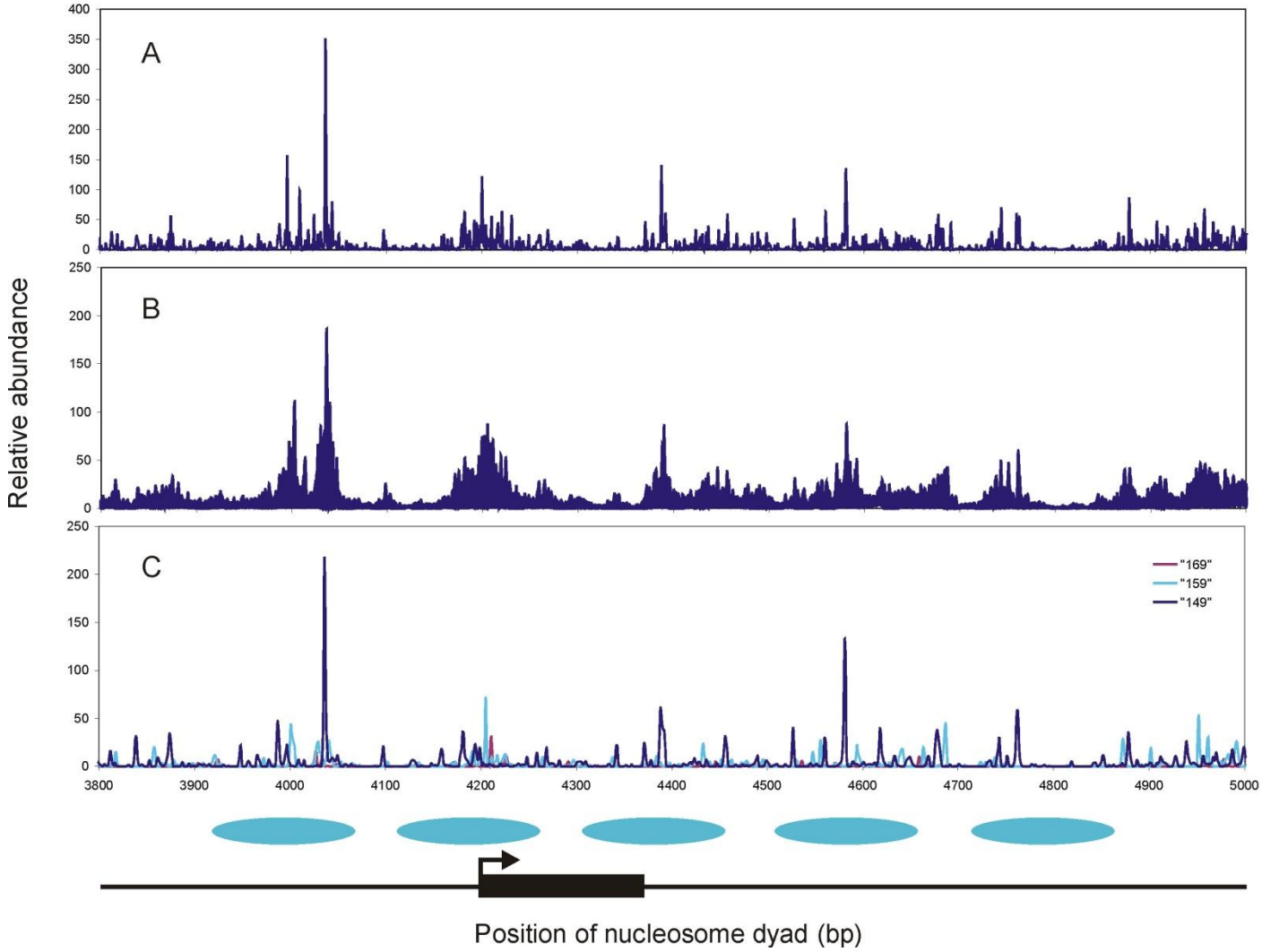
In vivo

Promoter organisation reminiscent of results from whole genome analyses



Mavrich et al. (2008) Nature, 453, 358-362

Histone octamer positioning maps: The BLG promoter region

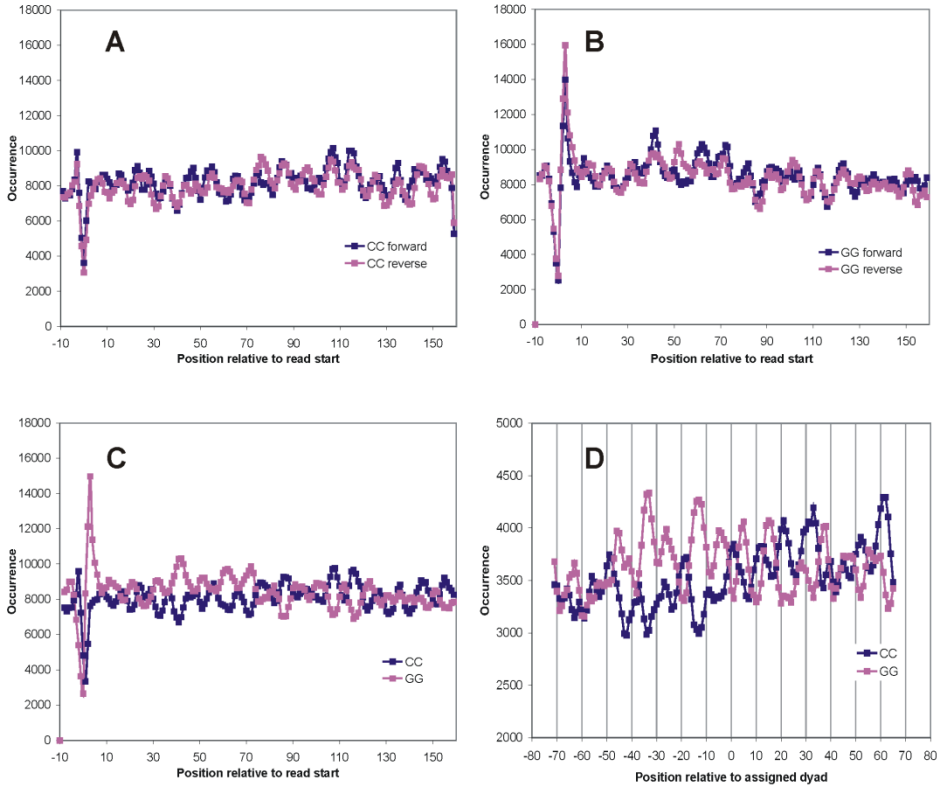


Method 1

Method 2

Method 3

In vivo

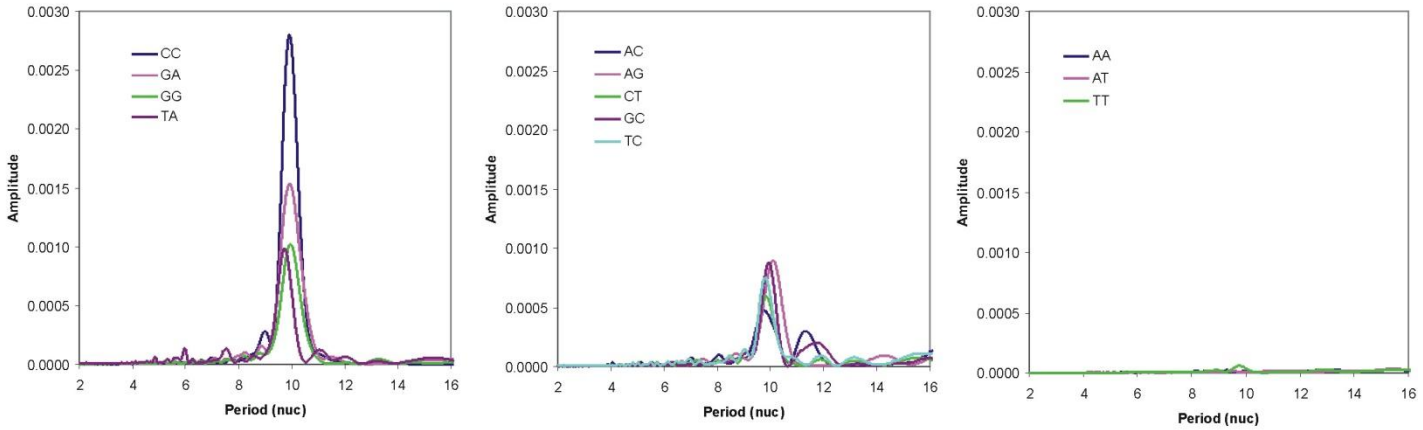


Sequence features of aligned reads

GG and CC
10 bp periodic
Out of phase by 5 bp

AA and TT not
periodically arranged

Fraser et al. (2009)
J. Mol. Biol. 390, 292-305



Mapping Nucleosome positioning sites by high-throughput sequencing provides a powerful comparative approach

- Other DNAs ✓
- Vary reconstitution conditions
  - Nuclease
  - Assembly reactions ✓
  - Add remodelling activities
- Vary histone octamer type
  - subtypes: H2Az, H3.3, CENPA ✓
  - Modified histones (acetylated, methylated etc) ✓
- Use modified (methylated) DNA

The influence of core histone type

Chicken (erythyrocyte)
Frog
Human
Yeast (S.cerevisiae)

Various DNA substrates as a mixture

In collaboration with Tom Owen-Hughes

Mapping Nucleosome positioning sites

(Illumina paired-end)

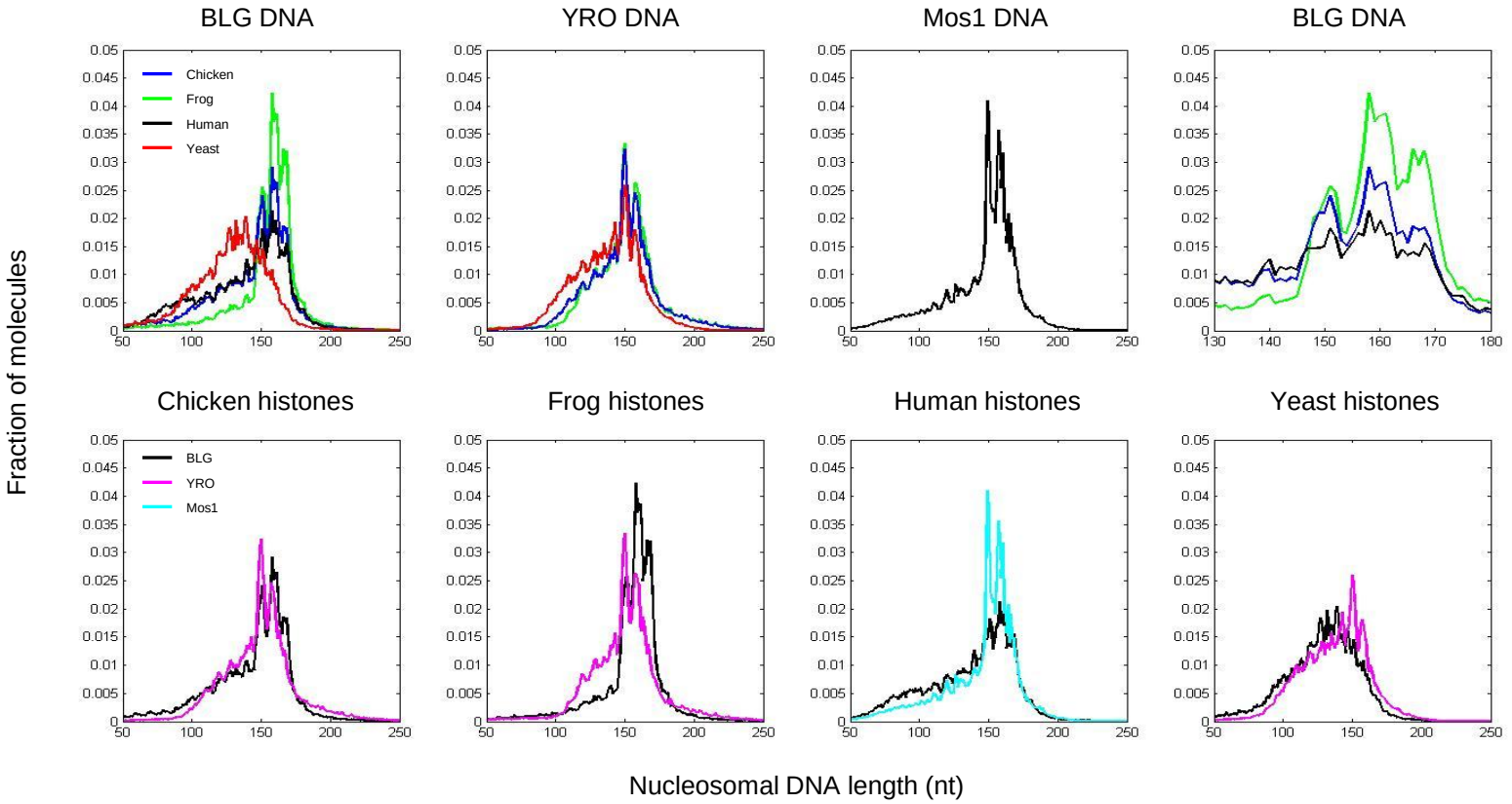
BLG DNA (12,861 bp)

4,400,000 reads (average)
Average read length ~ 145 bp

Coverage (per nucleotide)

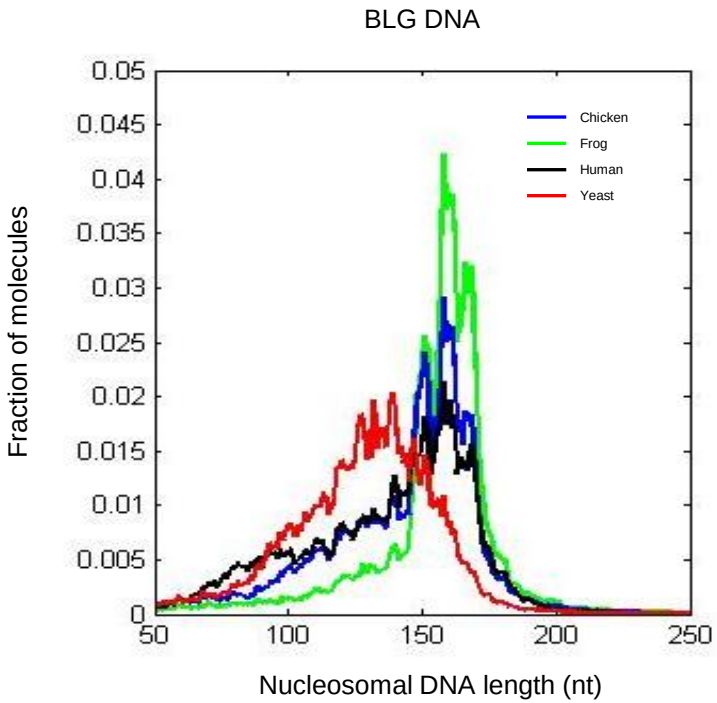
49,600 fold

Size distributions of histone octamer binding sites

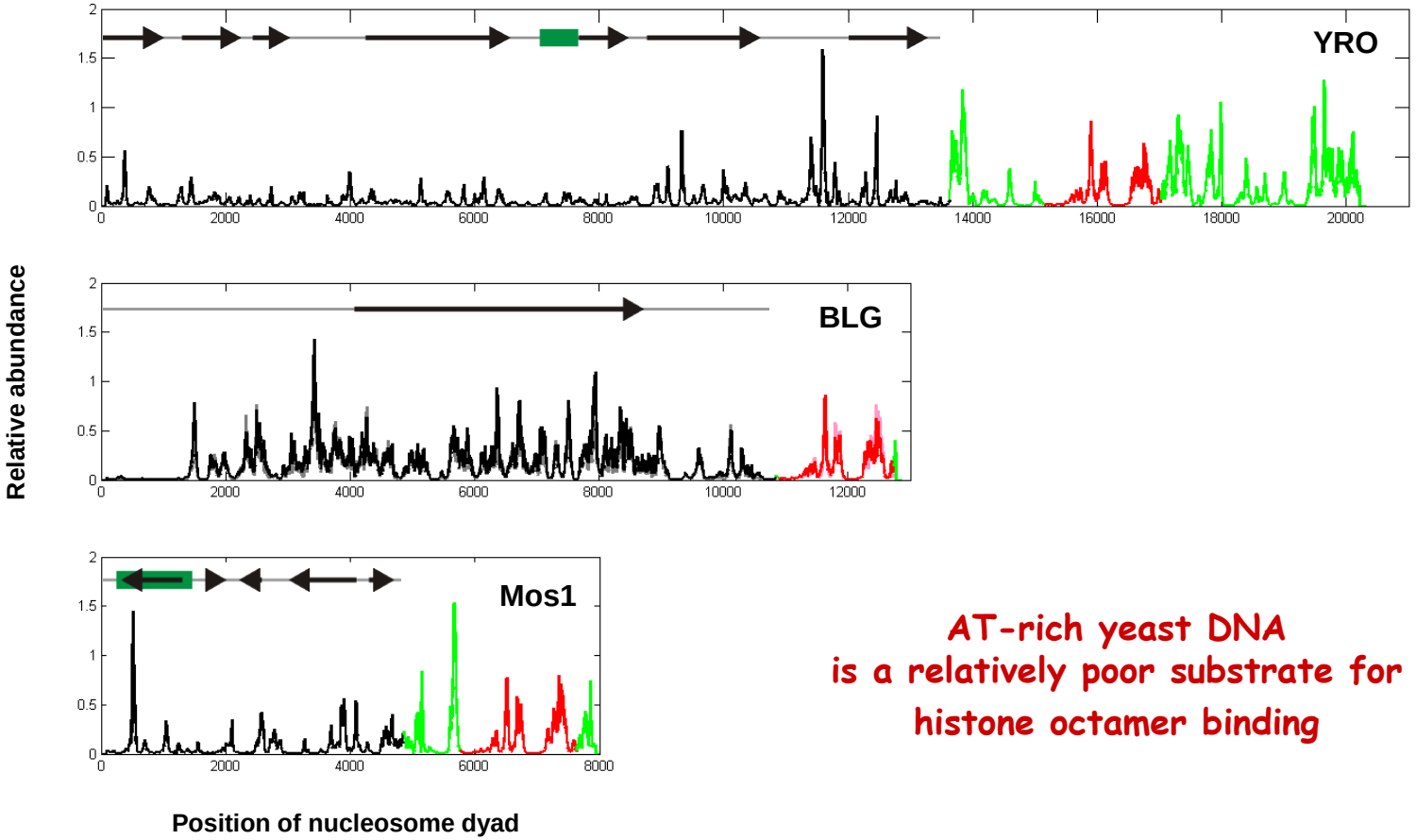


Size distributions of histone octamer binding sites reveal:

Quantized nucleosomal DNA lengths
Short yeast nucleosomal DNA lengths

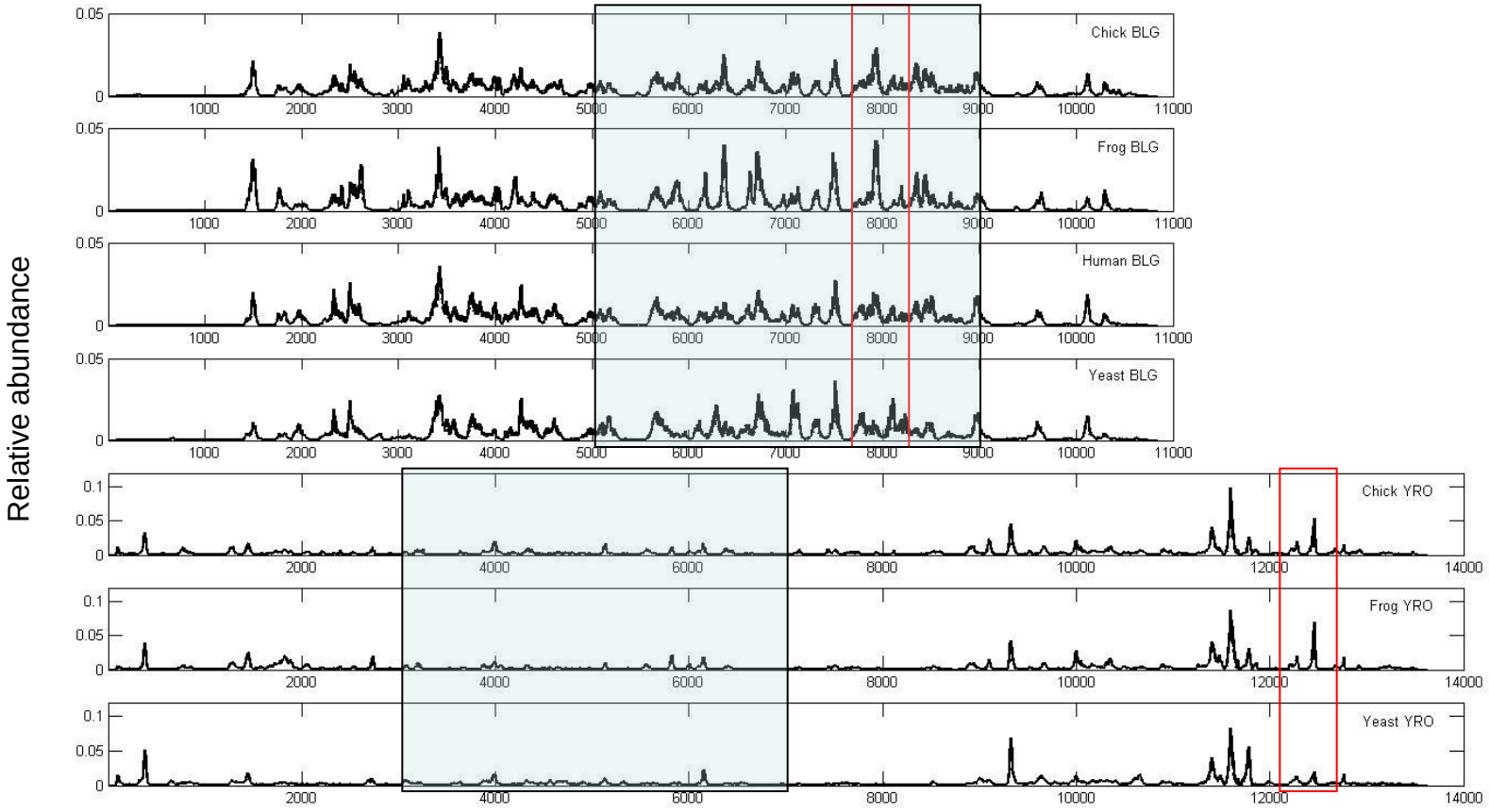


Core histone octamer positioning on genomic DNA sequences

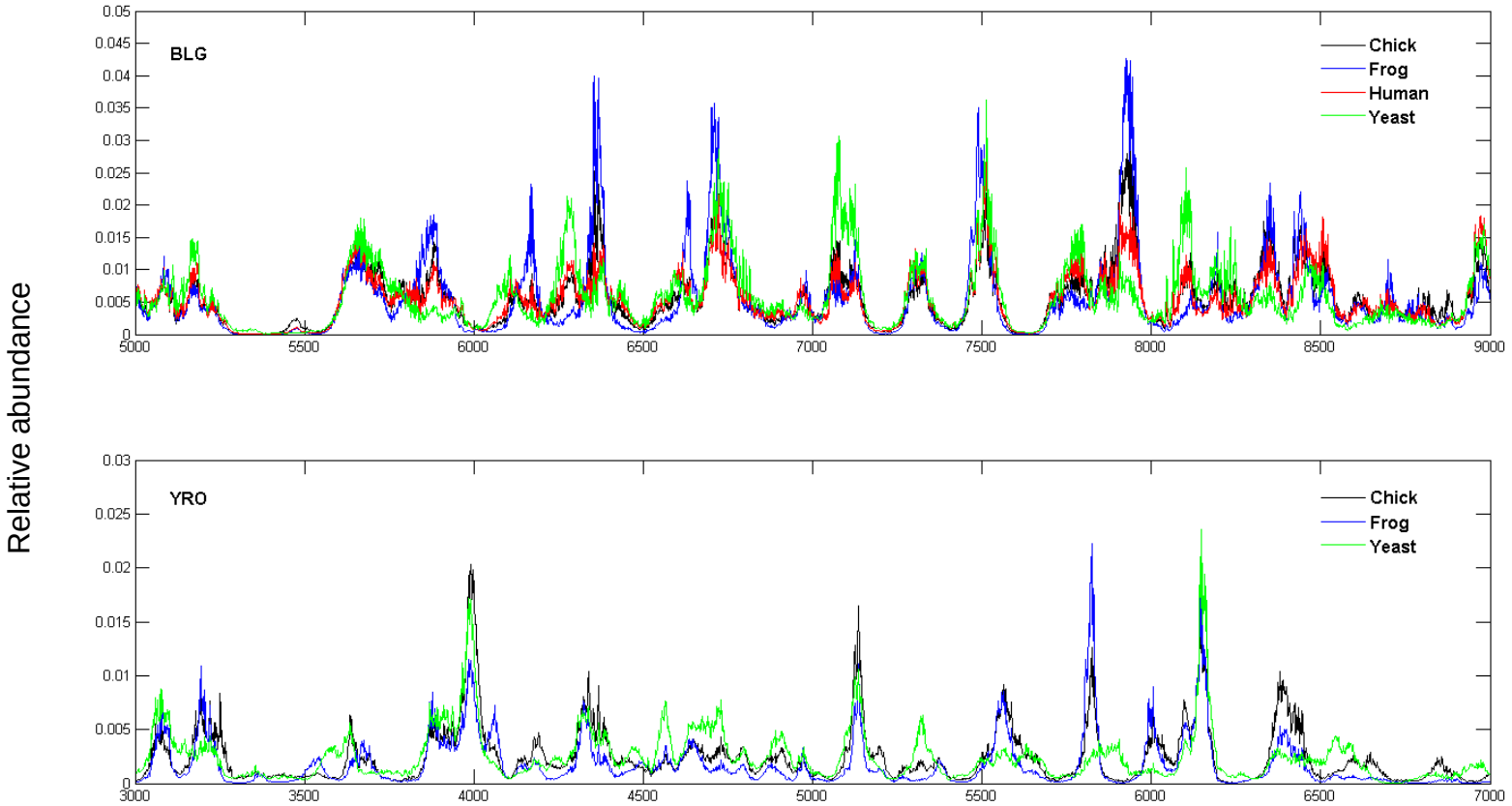


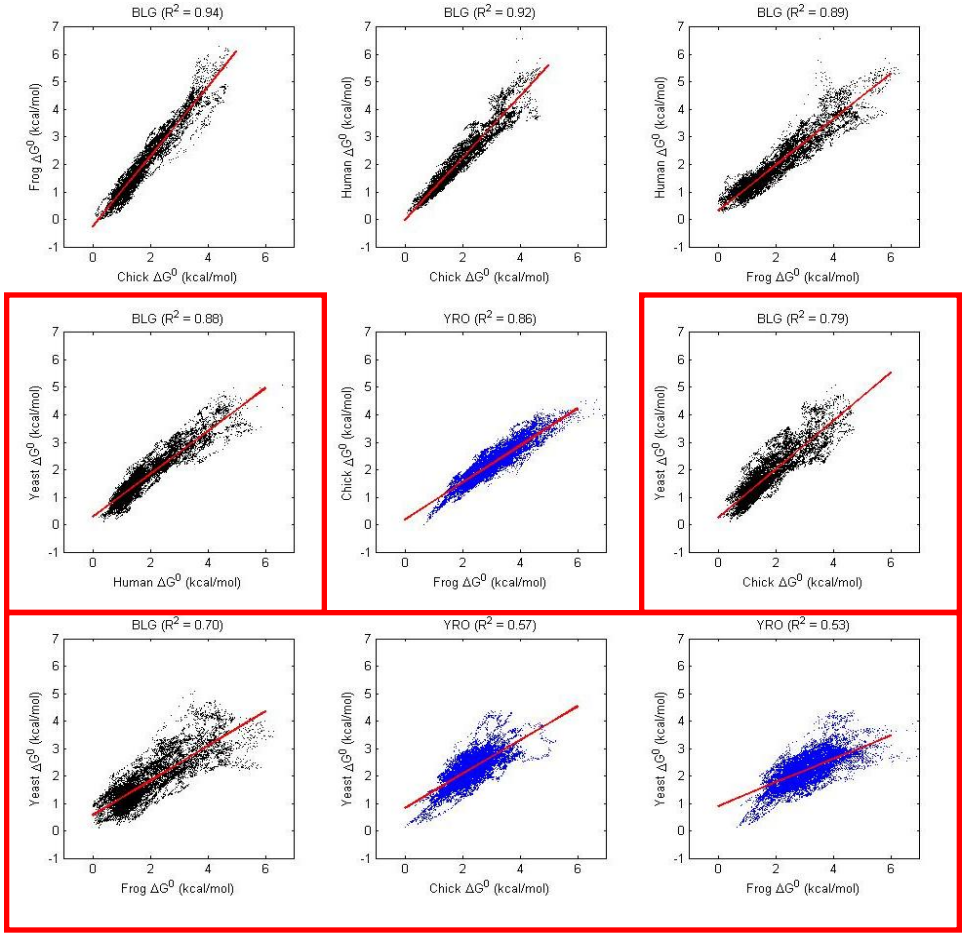
AT-rich yeast DNA
is a relatively poor substrate for
histone octamer binding

Nucleosome positioning is relatively independent of core histone type



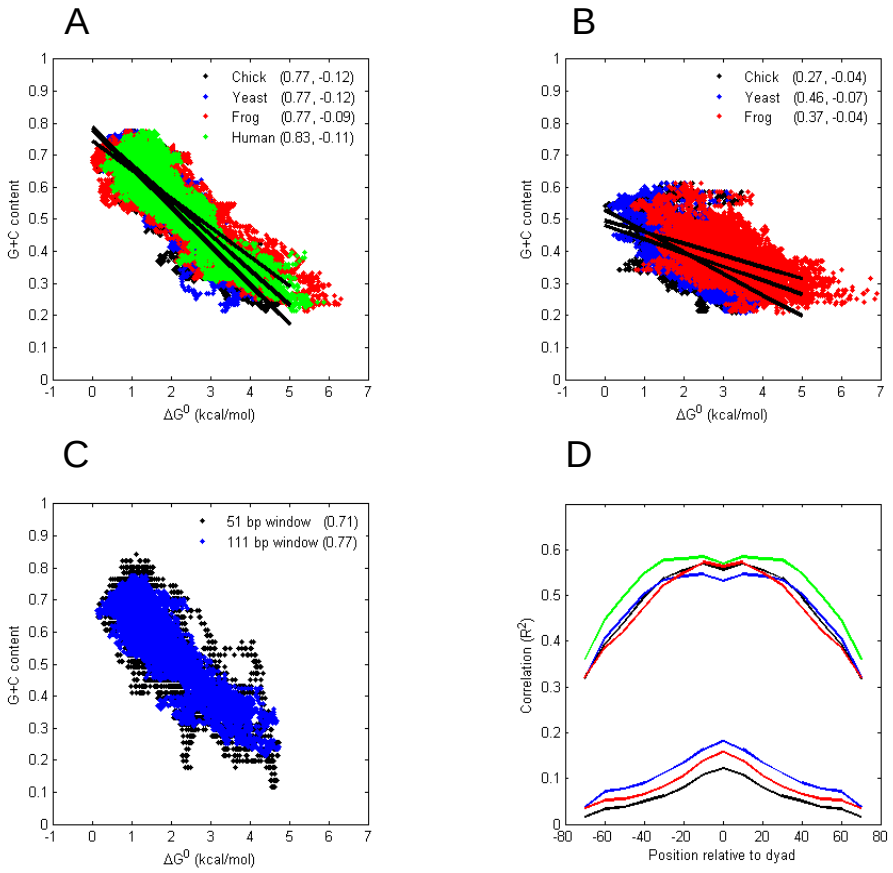
In some instances, nucleosome positioning displays histone octamer type-dependence





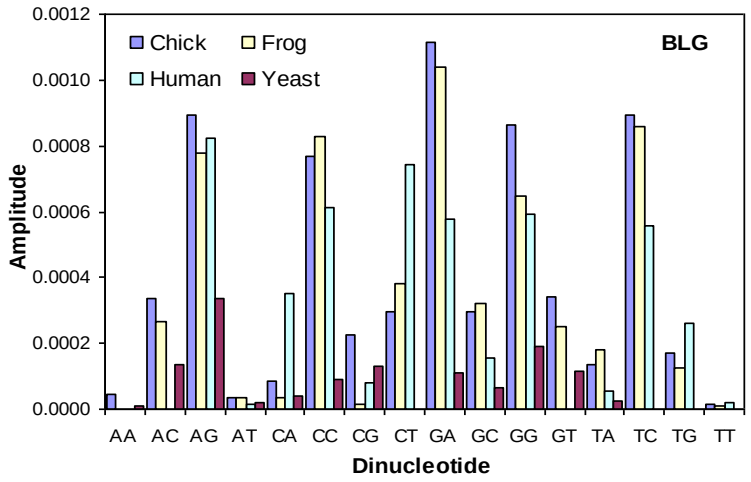
Relationship between histone octamer binding site maps

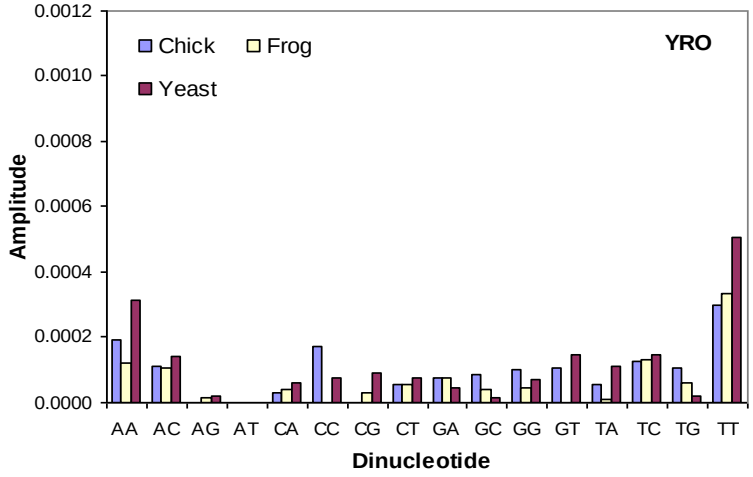
Scatterplots show that nucleosome positioning maps derived from yeast histone reconstitutes tend to be relatively poorly correlated to the chicken, frog and human histone maps



Sequence features of histone octamer binding sites

The affinity of the histone octamer for DNA is notably dependent upon the GC content of the binding site, a feature that is independent of histone octamer type.





Sequence features of aligned reads

Dinucleotide periodicities readily detected in DNAs from nucleosomes formed with chicken, frog and human histones are poorly represented in nucleosomes formed with yeast histones

On the AT-rich yeast DNA (YRO) AA and TT are the most notable periodically arranged dinucleotides

Although most core histone octamer types show only subtle differences in nucleosome positioning, the yeast histone octamer is most divergent in this context

Contributors

Ross Fraser
David Keszenman-Pereyra
Martin Simmen

Tom Owen-Hughes (Dundee)
Richard Meehan (Human Genetics Unit, Edinburgh)
David Finnegan (Edinburgh)
Kevin Docherty (Aberdeen)

BBSRC/Wellcome Trust/MRC

(Edinburgh)