My Studies of the Nucleosome Linker

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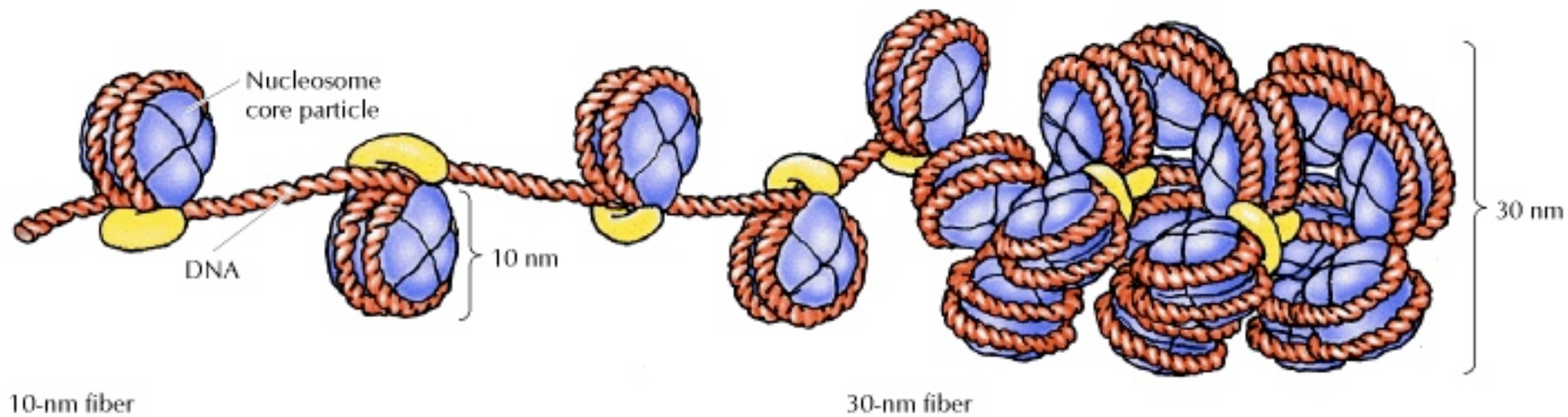
DNA, Nucleosomes, and Chromatin

- Nucleosome core particles are composed of 147 bp of double-stranded DNA wrapping around two of each of the histones H2A, H2B, H3, and H4: the histone octamer.

- Histone H1 is found on nucleosome where the DNA begins and ends its wrapping.



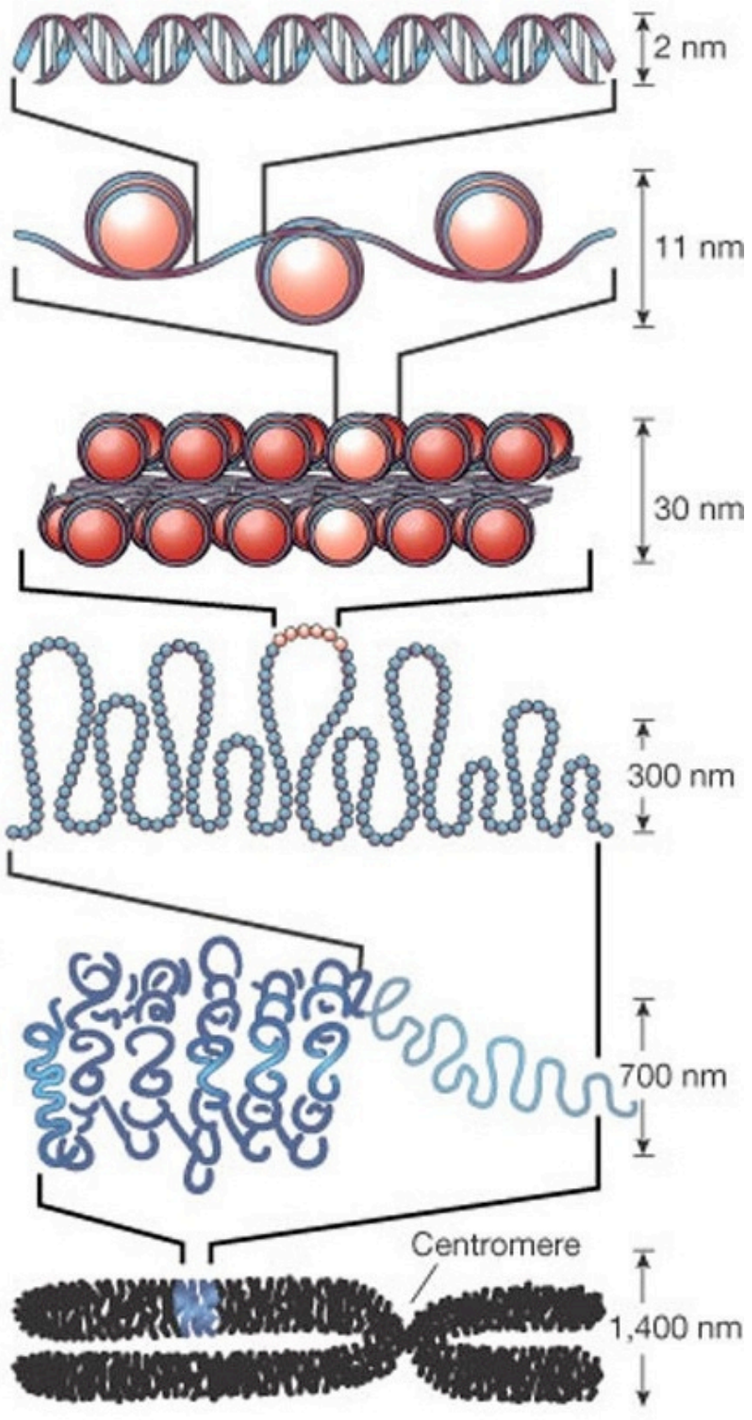
DNA, Nucleosomes, and Chromatin

- Chromatin is composed of the nucleosome core particles, and nucleosome linker DNA.

- The higher-order structure of chromatin includes the “30 nm fiber”.

- The 30 nm fiber forms loops and compacts itself in a remarkable way such that it isn’t entangled.

- At the highest level of organization, there is the mitotic chromosome itself.



(Felsenfeld & Groudine, 2003)

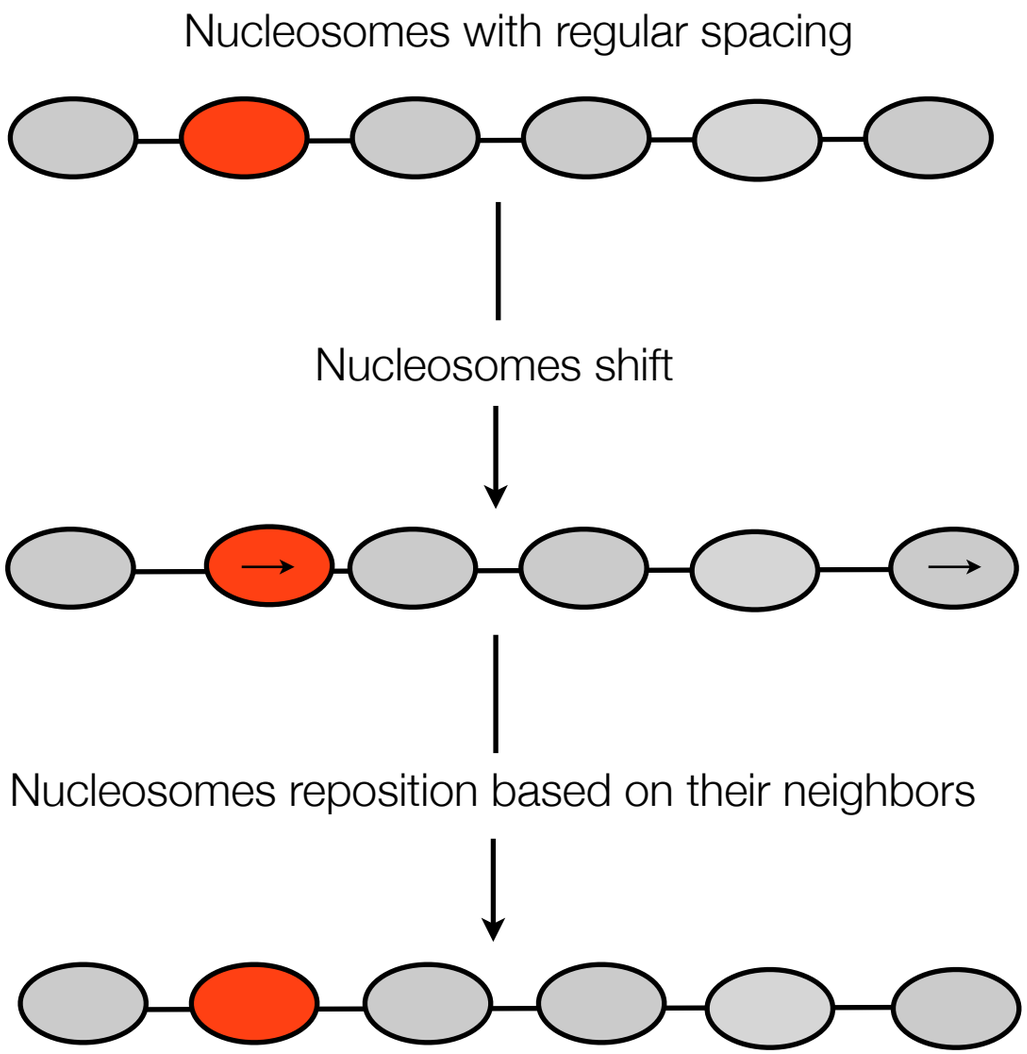
Nucleosome positioning

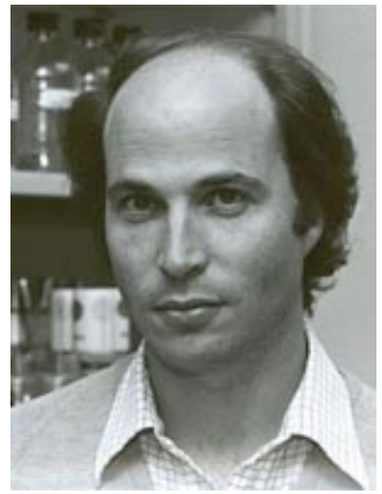
- The goal of nucleosome positioning is to precisely determine the loci of nucleosomes on the DNA molecule.

  Why bother?

- Nucleosomes play a role in gene regulation. The most simple example of this would be that the nucleosomes interfere with transcription.

Nucleosome Positioning





- Roger Kornberg first proposed the idea in 1981 that nucleosomes are “statistically” positioned on DNA.

- Most nucleosomes are weakly positioned, but have positions that are influenced by strongly positioned nucleosomes.

Nucleosome Positioning

- Alternate hypotheses concerning nucleosome positioning include “intrinsic” positioning.

- Intrinsically-positioned nucleosomes depend on the base sequence or composition.

- Defining the sequence/composition characteristics of intrinsic positioning is one of the main goals of Eran Segal’s work in nucleosome positioning.

Approaches to nucleosome positioning

- ChIP'ing (chromatin immunoprecipitation) core histones, followed by microarrays or high-throughput sequencing (ChIP-seq).

- Sequence/structural analysis.

- MNase-seq: digestion of nucleosome linker DNA using micrococcal nuclease, followed by sequencing of the remaining DNA (the DNA surrounding the nucleosomes).

MNase-seq

Chromatin

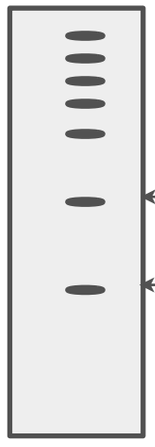
Digested Chromatin

Digestion with MNase

Digestion with proteinase, purify DNA

DNA fragments

Separate fragments with electrophoresis



dinucleosome DNA

mononucleosome DNA

Cut desired band, purify DNA

Optionally hybridize to selection beads.

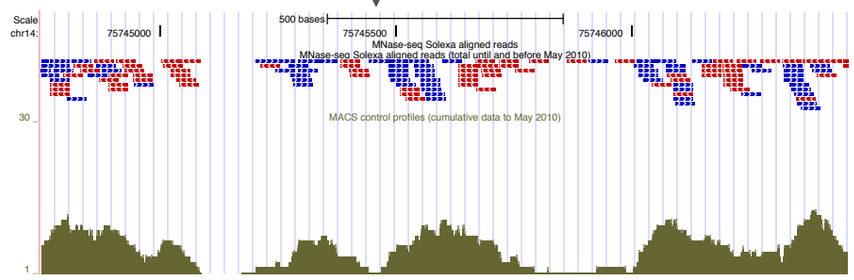
Deep-sequencing

Collect reads

Files containing reads

```
AAAGATTCATTTCCTGAGTCACTCTCCACAACTGAATTAA
TCGAACCCATGGCAAAGAAGCTAAAAACCTTGGAAAAAAA
AGGCATGAAGATGAAAATACCTTCTATAACATGATAAAGA
GGTTCCATTGTCAAGTTTATCGCATGGGGCTCTAACTGTT
GAGCATGGGGGATGCAGGTGAAGACGGAGGTGAAAATGCA
```

Align reads to reference genome assembly, make nucleosome profiles, call peaks



Correlate with other genomic data

Analyze phasing, autocorrelate, etc

Results

Drawbacks to MNase-seq

- MNase isn't perfect. It prefers some linkers over others, and occasionally cuts the DNA wrapping the nucleosome.

- Many many reads are needed for each experiment.

Histone H1 variants

- There are 6 main variants: H1.0 - H1.5.
- H1.1 is specific to certain cell types but the others are present in all cells and have varying levels of expression.
- Single knockouts in mice develop normally, though with positive and negative effects on gene expression.

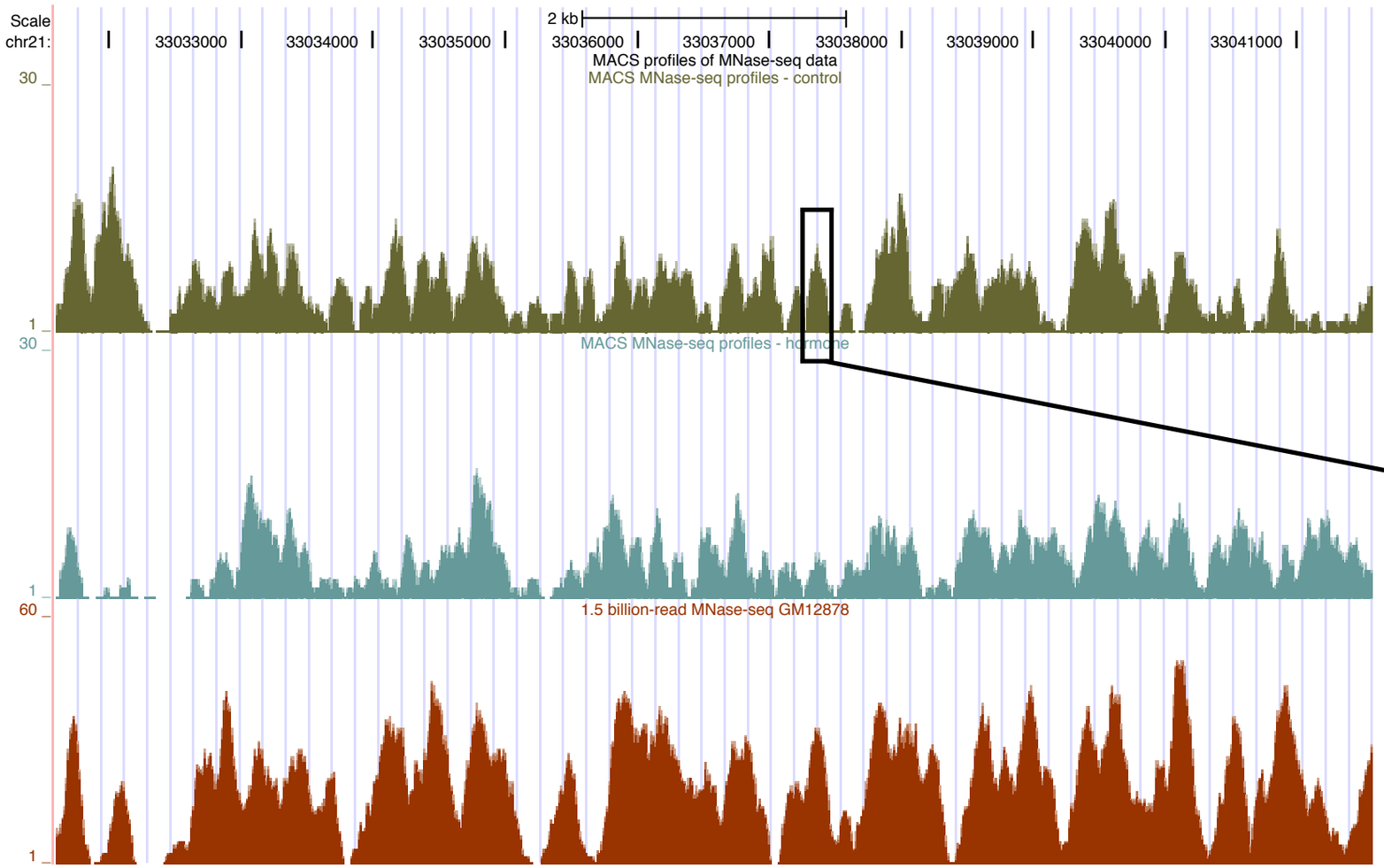
Histone H1 variants

- 5 T47D-derived (breast cancer) cell lines were established with each H1 variant tagged with the HA epitope. These were H1.0, H1.2, H1.3, H1.4, and H1.5.

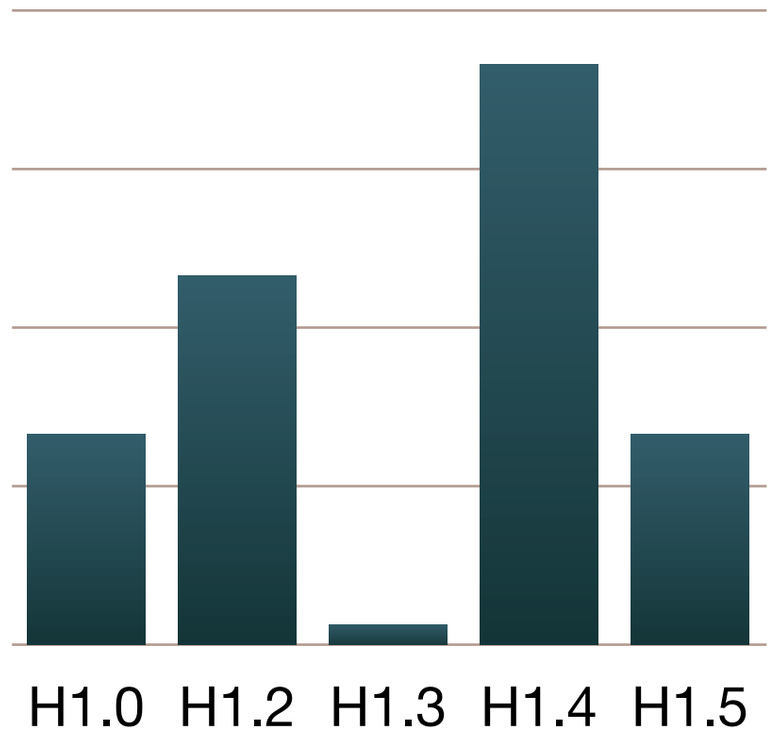
- Instead of performing ChIP with antibodies for each isoform separately (the currently available antibodies do not work well enough for ChIP), each ChIP uses the same HA antibody.

H1 variant	PAR binding domains	PARylation site	Cdk2 Serine consensus	Cdk2 Tyrosine consensus	Chromatin affinity	Chromatin condensa-tion	H2A/H2B dimer eviction
H1.0	aa181-194	aa3 aa115		aa119 aa141 aa153	Medium	Medium	Yes
H1.1		aa3	aa184	aa152	Low	Low	No
H1.2	aa19-30 aa147-158	aa3 aa16 aa115	aa174	aa31 aa147 aa155	Medium	Low	No
H1.3	aa14-26	aa3 aa16 aa115	aa190	aa18 aa147 aa155	High	Medium	Yes
H1.4	aa14-26	aa3 aa16 aa115	aa173 aa187	aa18 aa147 aa155	High	High	Yes
H1.5	aa161-172	aa3 aa16 aa115	aa190 aa18 aa173	aa138 aa155	High	Medium	Yes

Goal for H1-variant ChIP-seq



- Want to see differential levels of the variant H1 histones in some of the nucleosomes.

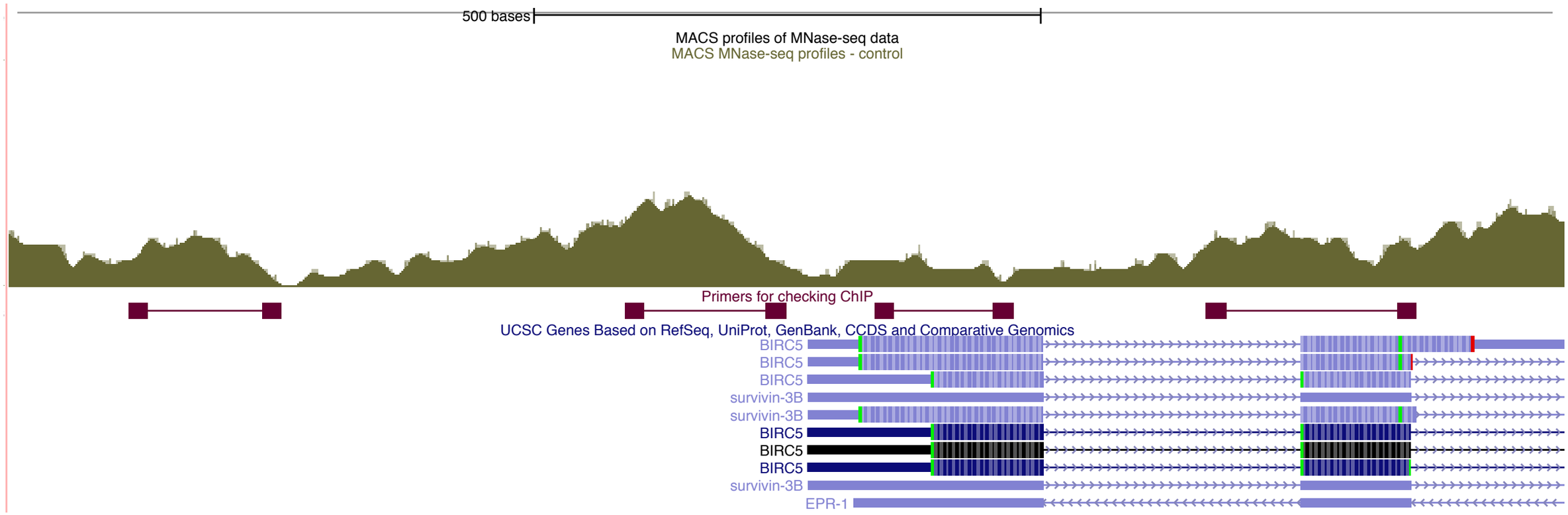


To-do before the ChIP-seq

- Before the sequencing, I need to find (fish) for individual nucleosomes that show differences in their H1s by qPCR-ing all over the place.

- To guide this fishing, I am first looking in the nucleosomes of the regulatory regions of genes associated with increased expression (according to microarrays) in cells with a Dox-induced H1.2 knockdown shRNA.

Fishing for differential H1



- I made four primers for BIRC5 aka survivin.
- BIRC5 was the most under-expressed gene when knocking-down H1.2.

More to-do before ChIP-seq

- Possibly re-do ChIP, and instead of sonicating the chromatin to be 200-300 bp, use MNase and obtain mononucleosomes.

- Create a T47-D cell line stably expressing HA-tagged histone H3.
    - I already have the HA tag and the H3 histone in plasmids at least.

- More fishing with qPCR.

Linker (Psora-seq) idea