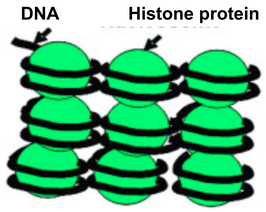
ChIP-Seq: genome wide analysis of histone 3 methylation in undifferentiated and differentiated mouse muscle cells

Ita and Xiaolan Zhang

Broad Institute of MIT and Harvard, Cambridge, MA, USA

Introduction

Histones are proteins around which DNA binds, and they provide the chromatin with its first level of structure. They have a high proportion of the amino acids arginine and lysine, which are positively charged, allowing them to bind the DNA, which is negatively charged. They can be modified by methylation, which can have either a positive or negative effect on transcription. Changes in the methylation of histones help regulate processes that require a change in transcription such as development. Methylation occurs on lysine and arginine residues most commonly on the histone tails of H3 and H4. Certain modifications (H3 K4 methylation) are strongly indicative of transcriptional activation, whereas other methylation events (H3 K27) are correlated with transcriptional repression.



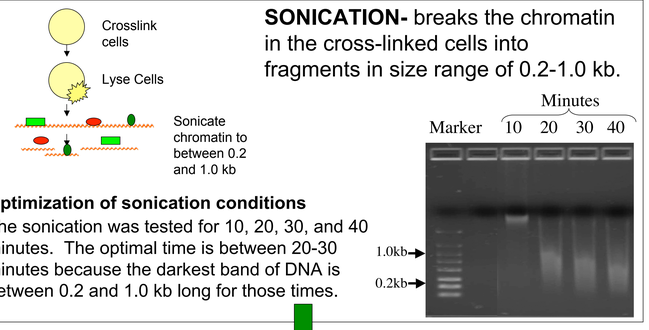
Chromatin immunoprecipitation (ChIP) is a powerful tool for identifying proteins, including histone proteins and non-histone proteins, associated with specific regions of the genome by using specific antibodies that recognize a specific protein or a specific modification of a protein *in vivo*. When the ChIP technique is combined with the next-generation sequencing technology, Solexa, that is capable of producing tens of millions of sequence reads during each instrument run, it enables us to quickly answer genome-wide questions.

To obtain a global perspective on the physiologic roles of histone modifications, we have designed an experiment to analyze H3K4m3 patterns genome wide in the mouse muscle cell line C2C12, in both the differentiated and non differentiated cells.

Materials

- C2 C12 mouse myoblast and myotube cells. These are undifferentiated and differentiated mouse muscle cells.
- Two antibodies used were for histone 3 tri-methylation at lysine 4 (H3K4M3) and lysine 27 (H3K27M3)
- 7900HT fast Real Time PCR system
- Biorupter (sonicator)

Methods and Results

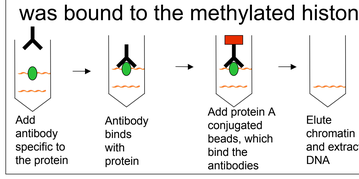


SONICATION- breaks the chromatin in the cross-linked cells into fragments in size range of 0.2-1.0 kb.

Optimization of sonication conditions

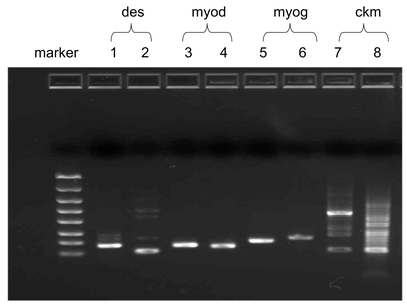
The sonication was tested for 10, 20, 30, and 40 minutes. The optimal time is between 20-30 minutes because the darkest band of DNA is between 0.2 and 1.0 kb long for those times.

IMMUNOPRECIPITATION- allows for isolation of the DNA which was bound to the methylated histones



Add an antibody specific to the tri-methylated histone. This will bind with the protein so the DNA can be isolated for qPCR.

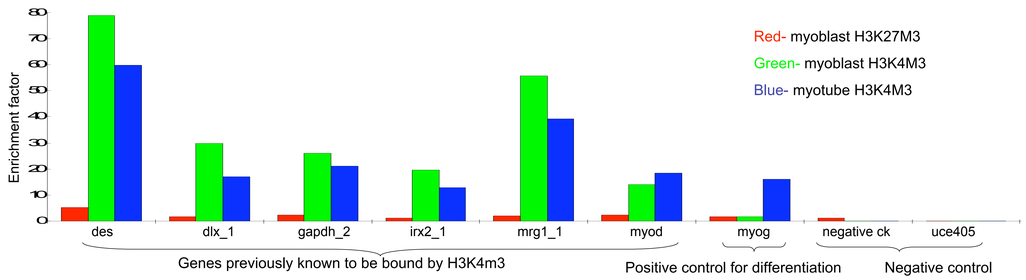
PRIMER DESIGN- designs primers for genes that will be used in qPCR



Primer test in PCR

Generally two pairs of primers are designed for each gene. The primers are tested using mouse genomic DNA as a template for PCR. The best primers are the ones with single band (lanes 3,4,5,6).

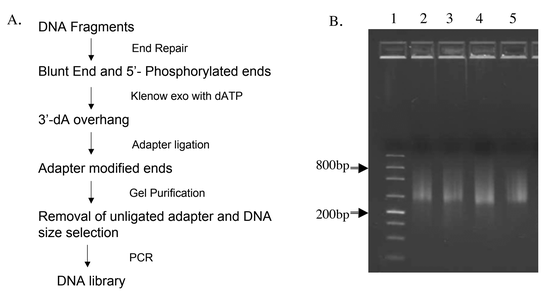
qPCR- tests the DNA quality and enrichment



Association of H3K4M3 with gene activation

The qPCR results show many previously identified genes are enriched and their activation is positively associated with H3K4m3 but not with H3K27m3, e.g. *des, dlx, gapdh, irx2, mrg and myod.* The *myog* gene, a marker for differentiation of C2C12 cells, is enriched in myotube, but not in non-differentiated C2C12.

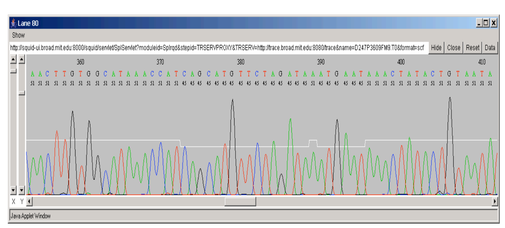
Solexa LIBRARY CONSTRUCTION



Solexa library preparation

A. Library construction procedure. B. H3K4m3 antibody enriched myoblast (lane 2), myotube(lane 4) and corresponding non enriched control (lane 3, lane 5). Lane 1 is marker.

SOLEXA SEQUENCING and data analysis- Solexa will sequence the DNA library, and data analysis will show H3K4m3 regulation patterns genome wide in the mouse muscle cell line C2C12



Conclusion

It has been reported that methylated H3K4 is found in actively transcribed gene regions in higher eukaryotes and that the level of H3K4m3 is coupled with transcription. Our data show that genes previously known to be associated with H3K4m3 are indeed bound to that form of methylated histone H3, but not to H3K27m3. Thus our analysis confirms existing models of H3K4m3. Additionally, our data suggest that histone modifications in coding regions, as well as promoters, play a general role in transcriptional regulation. We also found that myog is more enriched in differentiated muscle cells than in non-differentiated myoblasts, as expected. Future analysis of our sequencing data should show more genes like myog, which have differences in the enrichment between differentiated and undifferentiated cells. This will revel more genes involved in the process of muscle differentiation.

Future Directions

Currently a large number of cells are needed for ChIP-Seq analysis. It is difficult to get a large yet pure sample of certain cell types. We will optimize the protocol for a smaller cell number.

Cross-linking probably blocks the interaction of proteins and antibodies, so that not all of the antibodies are able to be used in ChIP. Additional tests of different antibodies are needed before they can be used for ChIP.

The analysis of more types of cells will give a picture of the overall histone modifications to the genome. Additional analysis of different stages of cells will help us understand the process of differentiation.

Literature cited

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Acknowledgments

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