

Identifying regulatory elements using chromatin signatures

Review of: Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, Barrera LO, Van Calcar S, Qu C, Ching KA, Wang W, Weng Z, Green RD, Crawford GE, Ren B. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nature Genetics* 2007, 39(3):311-318

The basic organisational unit of chromatin is the nucleosome, which consists of DNA wrapped around a protein core containing two copies each of four histone proteins: H2A, H2B, H3, and H4 (1). Post-translational histone modifications, including acetylation, methylation, phosphorylation, ubiquitination, and ribosylation, appear to be involved in the regulation of gene expression (2). Histone modification patterns have been used as a tool to study chromatin structure (3) and identify the functional roles of various modifications (4,5). A number of studies have been performed that investigate chromatin signatures. Recent reports explore the role of chromatin signatures in cancer initiation and progression (6) and the prognostic significance of global histone modifications in esophageal squamous cell carcinoma (7) and gastric adenocarcinomas (8).

Chromatin signatures can also be used as a tool for the functional annotation of the human genome. This study by Heintzman *et al.* demonstrates that active promoters and enhancers are associated with distinct chromatin signatures and that these chromatin signatures can be used to predict regulatory elements in the human genome (9). Using chromatin immunoprecipitation followed by microarray analysis (ChIP-chip), the chromatin architecture along 44 human loci, totaling 30 Mb, of the human genome was investigated. This study determined the patterns of core histone H3 and five histone modifications, including monomethylated Lys4 of histone 3 (H3K4), dimethylated H3K4, trimethylated H3K4, acetylated H3K9/14, and acetylated H4K5/8/12/16. This study also mapped four general transcription factors and nucleosome density to specifically identify chromatin features associated with promoters and enhancers.

Although the results of this study found that both promoters and enhancers have enrichment of histone acetylation, H3K4 dimethylation, and nucleosome

depletion, promoters and enhancers were distinguished by the methylation pattern at H3K4. Active promoters are marked by H3K4 trimethylation and enhancers are marked by monomethylation, but not trimethylation, of H3K4. These distinct chromatin signatures were used to develop computational algorithms to identify new regulatory elements and were used to predict 198 active promoters and 389 enhancers, including a novel functional enhancer for the carnitine transporter *SLC22A5* (also known as *OCTN2*). Functional validation studies were performed to assess the accuracy of the promoter and enhancer predictions. This involved the comparison of the prediction sets to a list of *in vivo* STAT1 binding sites, hypothesising that STAT1 sites are likely to occupy promoters and enhancers. The functional properties of the new promoter and enhancer predictions at STAT1 sites were validated using reporter assays. The results of the functional assays confirm the ability of the prediction model for identifying the location and function of novel promoters and enhancers.

Interestingly, when computational clustering was performed on the ChIP-chip profile data, four distinct classes of promoters, P1-P4, were observed. The expression of transcripts within each class generally increased from class P1 to class P4 (9). In addition, three classes of enhancers, E1-E3, were also identified. Another study has observed that the distinct chromatin signatures found at enhancers can distinguish the functional classes of enhancers in terms of transcription factor and coactivator binding (10).

Epigenetic maps of histone modifications have been developed, however, tools that search for functional elements using this epigenetic information have been lacking (10). Following the publication of this study, a learning method called ChromaSig was developed to find commonly occurring chromatin signatures in

tiling microarrays and sequencing data (10). Hon *et al.* applied this algorithm to nine chromatin marks across a 1% sampling of the human genome was able to identify five distinct chromatin signatures that correspond to known patterns associated with promoters and enhancers (10).

Heintzman *et al.* have developed a strategy to identify regulatory elements on the basis of their epigenetic characteristics, independent of motifs or other sequence features. This approach to the prediction of regulatory elements will be valuable to the functional annotation of the human genome and will further our knowledge of how epigenetic factors and distal transcriptional regulatory elements contribute to human development and disease (9).

References:

1. Luger K, *et al.* Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 1997, 389:251–260
2. van Leeuwen F, van Steensel B. Histone modifications: from genome-wide maps to functional insights. *Genome Biology* 2005, 6:113
3. Lupien M, *et al.* FoxA1 translates epigenetic signatures into enhancer-driven lineage-specific transcription. *Cell* 2008, 132(6):958-70
4. Skalnikova M, *et al.* Distinct patterns of histone methylation and acetylation in human interphase nuclei. *Physiol Res* 2007, 56(6):797-806
5. Tsai HW, *et al.* Sex differences in histone modifications in the neonatal mouse brain. *Epigenetics* 2009, 4(1) [Epub ahead of print]
6. Shukla V, *et al.* Histone acetylation and chromatin signature in stem cell identity and cancer. *Mutat Res* 2008, 637(1-2):1-15
7. Tzao C, *et al.* Prognostic significance of global histone modifications in resected squamous cell carcinoma of the esophagus. *Mod Pathol* 2008, Oct 24 [Epub ahead of print]
8. Park YS, *et al.* The global histone modification pattern correlates with cancer recurrence and overall survival in gastric adenocarcinoma. *Ann Surg Oncol* 2008, 15(7):1968-76
9. Heintzman ND, *et al.* Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nature Genetics* 2007, 39(3):311-318
10. Hon G, Ren B, Wang W. ChromaSig: a probabilistic approach to finding common chromatin signatures in the human genome. *PLoS Comput Biol* 2008, 4(10):e1000201