A BAYESIAN SYSTEM FOR MODELING PROMOTER STRUCTURE: A CASE STUDY OF HISTONE PROMOTERS

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LIST OF ABBREVIATIONS AND NOTATIONS

TFBS - Transcription factor binding site

TSS - Transcription start site

TF - Transcription factor

DPM - Dragon promoter mapper

NCBI - National Center for Biotechnology Information

EMBL - European Molecular Biology Laboratory

DDBJ - DNA Data Bank of Japan

DNA - Deoxyribonucleic acid

RNA - Ribonucleic acid

mRNA - Messenger RNA

IHGSC - International Human Genome Sequencing Consortium

bp - Base pair

A, C, G, T - Nucleotides/bases

PWM - Position weight matrix

EM - Expectation maximization

HMM - Hidden Markov Model

H1, H2A, H2B, H3, H4 - Five histone classes

DAG - Directed acyclic graph

CPD - Conditional probability distribution

CPT - Conditional probability table

HOMD – Higher order motif definition

Mi - Motif at position i

Si - Strand at position i

L(i+1)_i - Mutual length between motifs at positions i and i+1

TP - True positive

FP - False positive

Se - Sensitivity

ppv - Positive predicted value

cc - Correlation coefficient

stdev – Standard deviation

P(C, S, R, W) - Joint probability of nodes C, S, R and W

P(C) - Marginal probability of node C

P(S|C) - Conditional probability of node S given C

P(W|S,R) - Conditional probability of node W given nodes S and R

P(R=T|W=T) - Probability of R being *True*, given that W is *True*

H_0 - A hypothesis.

$P(H_0)$ - Prior probability of H_0

$P(E|H_0)$ - Conditional probability of observing the evidence E given that the hypothesis H_0 is true.

P(E) - Marginal probability of E

$P(H_0|E)$ - Posterior probability of H_0 given E

MCMC – Markov Chain Monte Carlo

LIST OF PUBLICATIONS

- **R Chowdhary**, SL Tan, RA Ali, B Boerlage, L Wong, VB Bajic. Dragon Promoter Mapper (DPM): a Bayesian framework for modeling promoter structures. *Bioinformatics*, Apr 2006 (Epub ahead of print). PMID: 16613910.
- **R Chowdhary**, L Wong, VB Bajic. Finding functional promoter motifs by computational methods: a word of caution. *International Journal of Bioinformatics Research and Applications (IJBRA)*, accepted.
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- V Narang, **R Chowdhary**, A Mittal, WK Sung. Bayesian network modeling of transcription factor binding sites a book chapter in: *Bayesian Network Technologies: Applications and Graphical Models*, Idea Group Publishing, Pennsylvania, USA 2006.
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SUMMARY

Gene regulation has been recognized as an important line of research due to its crucial biological significance. Very little is known about gene regulatory mechanisms till date. One of the essential regulatory regions of the gene is its promoter region. Recognition and annotation of promoter regions besides other regulatory regions in the genomes remains a fundamental task even today. This is because the genomic data continue to stay largely unannotated, particularly the regulatory regions. One reason that can be attributed to this problem is that promoter recognition and annotation is an extremely challenging problem in part due to the complexity of the data involved.

Promoter modeling, a term used interchangeably with promoter recognition and annotation, can be performed using experimental techniques. However, due to the huge size of genomic data involved, computational techniques have become a good compliment alongside. Researchers in the past have proposed many computational promoter modeling approaches, most of which have primarily been focused towards *general* promoter recognition. However, these programs not only generally suffer from high number of false positives but also appear too general to faithfully model all classes of promoters together. Promoters of different classes generally have too little in common to be described by a single promoter model. Another type of programs that perform better are *specific* promoter recognition programs, which focus on modeling a particular class of promoters. Still, *specific* promoter recognition approaches have received relatively less focus compared to general promoter recognition programs, perhaps due to unavailability of sufficient, relevant and clean data of different classes of promoters. The present study is an attempt in this direction. My PhD project is aimed at modeling and recognition of specific promoter structures, which has till date received only partial success. I have focused explicitly on histone protein-coding genes. Histones are an important class of

proteins that play a crucial role in various cellular functions related to gene transcription and regulation.

I have proposed a novel computational methodology based on Bayesian networks to model promoter structures of histone genes based on the properties of regulatory signals present in them. Using the developed histone promoter model, my methodology attempts to discover the regions in the human genome that have structures similar to histone promoter model; such regions may in part represent promoters of the genes that may potentially be coregulated with histone genes. My methodology is a general-purpose framework to model promoter structures of any class of genes. The methodology has been shown to perform better than several other similar well-known programs. It has certain distinct advantages compared to the other related systems that have been highlighted in the text. The results obtained in this study have been found to be statistically significant and have been validated with experimental data.

To the best of my knowledge this is the first comprehensive study that has attempted to systematically computationally model histone promoter structures. Overall, the present study has resulted in the development of, i) Dragon promoter mapper (DPM), a tool to model promoter structures of a particular class of genes, and ii) annotated data of histone promoter models, that compliments just a handful of datasets known to the research community for which specific promoter models have been studied, and iii) data of human genomic regions that have similar structures as histone promoters.

I hope these tools and data would prove to be useful to the research community.

1. INTRODUCTION

Biological studies can be performed by experimental wet-lab techniques. However, these techniques can be very expensive and time consuming. The experimental techniques therefore are not suited to handle huge amounts of genomic data, such as those that are present in the public databases of NCBI (http://www.ncbi.nlm.nih.gov/), EMBL (http://www.ebi.ac.uk/embl/) and DDBJ (http://www.ddbj.nig.ac.jp/) and others. Thus, there is a need for computational techniques that can be applied on the large genomic datasets, with the aim to verify the results so obtained by experiments later. Such pragmatic considerations have introduced the field of Bioinformatics.

Bioinformatics has been established in the last 20 years as one of the most interdisciplinary fields of scientific and technological research that involves several disciplines such as computer science, molecular biology, genetics, and chemistry among others. Loosely speaking, bioinformatics attempts to provide answers to biological questions based on computational analysis of biological data. To make efficient bioinformatics solutions there must be a successful synergy between,

i) biological background understanding of the problem,

ii) biological data understanding,

iii) data conversion into forms appropriate for modeling of the underlying problem, and

iv) computer science type of solution to the problem.

This is why it is sometimes difficult to make strict boundaries between biology and computer science. From the viewpoint of computer scientists it is of interest to expand the current application domains of the existing technologies to new and exciting areas of life sciences. This study represents a step in this direction, attempting to apply a computer science technology to a difficult yet exciting functional genomics problem of gene regulation.

The difference between man and monkey is gene regulation. - by Leroy Hood (quoted in Werner 2001).

The above quote highlights the importance of gene regulation in the very existence of life forms. Still, much is unknown about it in general. Gene regulation is a complex mechanism that determines which all genes would express in a particular cell at a particular time and by how much. Such differential gene expression characteristics are essential for normal functioning of cells in an organism. Though there have been many studies in the past to computationally unravel gene regulatory mechanisms, this field is still wide open and much work needs to be done. A crucial player in gene regulation, that has been the focus of many gene regulation studies, is the promoter region of the gene. Promoter is a regulatory region on the DNA that covers the start of the associated gene which is known as transcription start site (TSS), and contains a set of "switches" or transcription factor binding sites (TFBSs) where particular proteins or a combination of proteins known as transcription factors (TFs) interact in a specific manner and regulate the initiation of gene expression process temporally and spatially in the body.

Promoter modeling has been recognized as an important line of research (Fickett and Hatzigeorgiou 1997, Werner 1999, 2003) due to its crucial biological significance. However, due to a variety of reasons as highlighted later in the text, promoter modeling is an extremely challenging problem. Researchers in the recent past have commonly employed computational tools to perform promoter modeling which largely involves characterization and recognition of promoters. While characterization involves annotating the structures and the associated regulatory functions of known promoter sequences, recognition of promoters involves detecting previously unknown promoter sequences from across the genomes. In characterization, for example, programs have been built that discover TFBSs and other structurally and functionally important

signals in the promoter sequences. Then there are sequence alignment programs that are used to detect homology between input promoter sequences by aligning them multiply (Higgins et. al. 1994) or in pairs (Altschul et. al. 1990). Promoter recognition programs, on the other hand, aim to search for novel promoters from across various genomes. These programs have often exploited the fact that promoters cover the TSSs of their respective genes. A novel promoter detected from the genome may potentially help in gene discovery. The motivation behind promoter modeling is therefore usually characterization/annotation of genome data. Genome data remain largely uncharacterized even today, particularly with regard to annotation of regulatory regions such as promoters and their functions. The reason for this may be attributed to the complexity of the problem. For example, human genome comprises 3 billion base pairs and genes and their regulatory regions are believed to form a very small fraction of this number. Thus, the problem is like searching a needle from a haystack.

Based on the objectives, promoter modeling techniques can be divided into two broad categories, namely, *general* promoter modeling and *specific* promoter modeling. General promoter modeling focuses on building computational tools to model all promoters together, while, specific promoter modeling focuses on building computational tools to model particular class of promoters. For example, general promoter modeling may involve building models based on general promoter structure properties of all known promoters together, while specific promoter modeling may involve building models based on promoter structure properties of a class of promoters, such as muscle specific gene promoters. Models built on both techniques can be used to scan the genome and recognize putative promoters that match the promoter properties defined by the models. Based on these two techniques, many computational strategies have been proposed in the past to recognize putative promoter regions of DNA (Fickett and Hatzigeorgiou 1997, Werner 1999, 2003, Pedersen et. al. 1999), however these programs have generally suffered from high number

of false positives. The fact is that at this moment there is no computer program which can predict eukaryotic promoters very efficiently (Bajic and Seah 2003a).

Relatively, specific promoter recognition programs show better specificity compared to general promoter recognition programs (Werner 1999). Still, specific promoter recognition programs have received relatively less focus compared to general promoter recognition programs, perhaps due to unavailability of sufficient, relevant and clean data. Apparently, building a single methodology catering to all types of promoters together appears not only *too general* but also highly complex and unrealistic. Various promoter sequences have too little in common to be described by a single promoter model. A more prudent yet challenging approach is to thus focus on methodologies that address specific classes of promoters. Additionally, there are other advantages of *specific* promoter recognition programs over *general* promoter prediction programs, such as in (i) determining the tissue specificity of genes, (ii) predicting the function of genes, and (iii) identifying co-regulated genes. Such information is presently available for only a very small fraction of genes.

My PhD research project is aimed at the problem of modeling and recognition of specific promoter structures, which has till date received only partial success. The project involves developing a methodology to model promoters of any particular class of genes. I have focused explicitly on human protein-coding genes, and within this broad class on a special group of genes which produce histone proteins. Histones are an important class of proteins that play a crucial role in various cellular functions related to gene transcription and regulation. This focused approach allowed me to utilize specific properties which many of the promoters of this class share.

I have proposed a novel computational methodology to model promoter structures of histone genes based on the properties of regulatory signals present in them. Using the developed histone

promoter model, my methodology attempts to discover the regions in the human genome that are structurally similar to histone promoter model; such regions may represent promoters of the genes that are potentially co-regulated with histone genes.

I have used Bayesian networks to model histone promoter structure, though there could possibly be many other approaches. Bayesian networks offer a natural way to represent probabilistic data (Jensen 2001). As highlighted later in the text, biological data are prone to sequencing and annotation errors due to various reasons and histone promoter data are no exception. The errors in such data lead to uncertainties that can be aptly handled by the probabilistic framework of Bayesian networks.

To the best of my knowledge this is the first comprehensive study that has attempted to systematically computationally model histone promoter structures. The study has also attempted to discover genes across the human genome that are co-regulated with histone genes. To date there are only a handful of datasets known to the research community for which specific promoter models have been studied. These include the sets of i) glucocorticoid and heat-shock responsive genes (Claverie and Sauvaget 1985), ii) globin family promoters (Staden 1988), iii) muscle specific genes (Wasserman and Fickett 1998, Klingenhoff et. al. 2002), and iv) liver specific genes (Krivan and Wasserman 2001). This study contributes another well-annotated dataset to the research community. As highlighted later in Chapter 5, the DPM system that I have developed for modeling histone promoter structure has distinct advantages compared to the other related systems. DPM has shown better performance (Chowdhary et. al. 2006) in terms of sensitivity and specificity of promoter prediction. It can analyze multiple subtypes of promoter sequences within a given promoter class. DPM also allows the user to incorporate biological background knowledge in the model. Aside, DPM is not rigid and the user can flexibly develop and test his model according to his suitability. DPM methodology is generic and can be applied to model

promoters of any class of genes or co-regulated genes. Overall, DPM provides a robust methodology that can principally be applied for general purpose modeling of structures of any regulatory region including promoter.

My presentation is divided as follows: The biological background relevant to the problem in question is in Chapter 2 with sub sections on, i) Regulation of Gene expression and Promoter, ii) Difficulty in modeling promoters computationally, iii) Promoter modeling tools and resources. Chapter 3 discusses specific aspects related to research project such as histone basics and Bayesian networks. Chapter 4 introduces my PhD research problem and work done. The section on work done has sub sections of, i) Elucidation of histone promoter content, ii) Dragon Promoter Mapper (DPM) - a promoter structure modeling system, iii) Modeling of promoter structure of human histone genes using DPM, iv) Comparative analysis of DPM's performance and several other systems, v) Human genome scan using human histone promoter structure model. The thesis completes with a conclusion in Chapter 5.

2. BIOLOGICAL BACKGROUND

A eukaryotic organism contains the complete genome in the nuclei of most of the cells. The genome is the complete set of genetic information inherited from the parents and comprises all the genes. The genome is physically present in the form of a polymer called DNA (deoxyribose nucleic acid). The basic unit of DNA is a nucleotide which comprises sugar-phosphate backbone and one of the four bases adenine (A), cytosine (C), guanine (G) and thymine (T). The genetic instructions encoded in genomic sequences are very less understood. The human genome, for example, is extraordinarily complex. The protein-coding bases of its 30,000 genes span only less than 2% of the entire 3 billion base pairs long genomic sequence (IHGSC). Of the rest non-coding segment of the genome, another small part contains regulatory regions controlling the expression of these genes. Very little is known regarding these functional regulatory regions.

2.1 Regulation of Gene expression and Promoter

Genes in DNA act as a blueprint for the production of RNA and proteins (another polymer) inside the cells. Proteins play an essential role in cellular functions. A vast majority of genes are known to produce proteins as their end products. The process of synthesizing proteins in cells is known as gene expression. Gene expression involves transfer of sequential genetic information from DNA to proteins and broadly involves following stages (Fig. 2.1):

i) transcription, where a gene's DNA sequence is transcribed into a single stranded sequence of primary transcript or pre-mRNA.

ii) capping, where primary transcript is capped on the 5' end, which stabilizes the transcript by protecting it from degradation enzymes.

iii) poly-adenylation, where a part of 3' end of the primary transcript is replaced by a poly-A tail for providing stability.

iv) splicing, where introns are removed from the primary transcript to form messenger RNA (mRNA).

v) mRNA is transported from nucleus to cytoplasm.

vi) translation, where a ribosome produces a protein by using the mRNA template.

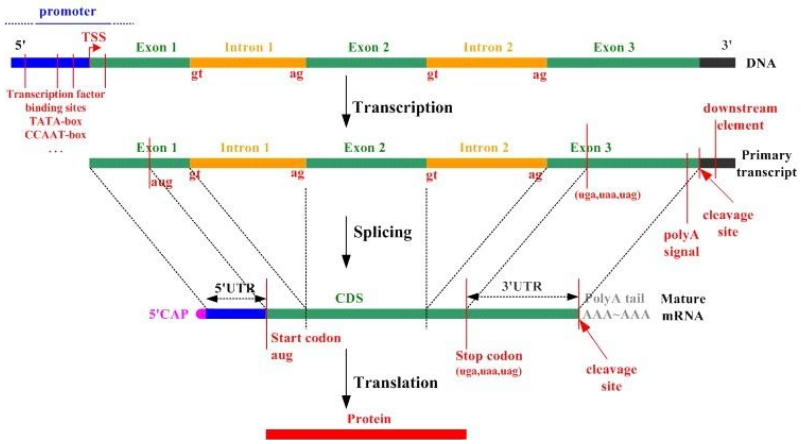


Fig 2.1: Stages of gene expression in cell *(courtesy: Professor Vladimir Bajic)*

Gene expression is a strictly regulated process in cells. The regulation of gene expression is important as it determines where (cell-type), when (developmental stage), how, and in what quantities various proteins are produced in cells. This decides how cells develop, differentiate and respond to external stimuli. The detailed mechanism of gene regulation, however, still remains unclear. Gene regulation occurs at various stages of gene expression from transcription to translation (stages shown above), though transcription is generally believed to be the most important stage. The transcription stage of gene expression involves regulatory DNA regions known as promoters.

Every gene has at least one promoter that mediates and controls its transcription initiation. This control mechanism occurs through a complex interaction between various TFs that get attached to

their specific TFBSs present in the gene's promoter region. A promoter is usually defined as a non-coding region of DNA that covers the TSS or the 5' end of the gene. Bulk of promoter region typically lies upstream of the TSS. The promoter region in Eukaryotes is usually difficult to characterize because of high variability. For example, promoters may vary from a few hundred bases in some genes to several kilo bases in the others. A promoter may be typically classified as,

i) Core promoter

- usually lies up to 30 bp upstream with respect to the TSS
- contains the TSS
- contains binding site for RNA polymerase
- contains general binding sites (i.e. binding sites commonly found in many promoter types)
- example of a binding site in this region is TATA-box

ii) Proximal promoter

- usually lies between 200 bp to 300 bp upstream with respect to the TSS
- contains specific binding sites that control temporal and spatial expression of a gene
- example of a binding site in this region is CAAT-box

iii) Distal promoter

- lies upstream of the proximal promoters, may be located thousands of bases away from the TSS
- contains specific binding sites that control temporal and spatial expression of a gene

Aside a promoter, there are some additional regulatory regions on the DNA that work cohesively with the promoter in regulating a gene at the transcription stage. These regions are usually located thousands of bases upstream or downstream of the TSS and regulate the rate of transcription of the associated gene. Alike promoters, the regulation here also occurs through specific regulatory TFBSs present in these regions. Examples of such regions include enhancers, silencers and boundary elements; enhancers increase the gene's transcription rate while silencers decrease it.

Promoter regions are interspersed with characteristic short TFBSs patterns (~6-20 bp in length) that provide functionality to these regions. These patterns are usually conserved across species and are degenerate in nature. As TFBS motifs are short they tend to occur frequently anywhere in the genome, however, only those that are present in the regulatory regions of the genome may be functionally active. TFBSs show large variations across promoters of a species; some promoters may have particular TFBSs that others do not have. Between promoters, TFBSs do not intrinsically have any bias towards a particular location or orientation (Werner 1999). However for a particular class of promoters such a bias may be observed (Wasserman and Fickett 1998). Adding to the complexity, the nature of function of a TFBS may depend on its context/location within the promoter. For example, the factor AP1 suppresses gene transcription when it binds to its binding site in the distal promoter, while it supports the transcription when it binds to its binding site in the core promoter (Werner 1999). Such contextual behavior of a TFBS may be dictated by factors such as, tissue specificity, and cell-cycle & developmental stage. Overall, there are large variations in TFBS distributions across promoters and their associated functions.

An existing paradigm is that within a promoter, TFBSs uniquely combine to form a module that imparts a specific functionality to the promoter. A typical functional module organization is shown in Fig 2.2. The module is characterized by its features, such as specific order of TFBSs,

their orientation, their location, and mutual distance between them. The module functions as a single cohesive unit and may not work if any of the module elements is absent or if any of its features gets disturbed. A module may be more specific on the DNA compared to a single TFBS. Due to this, modules are sometimes preferred over single TFBSs for modeling promoters. In this text I have used *promoter module* and *promoter structure* interchangeably.

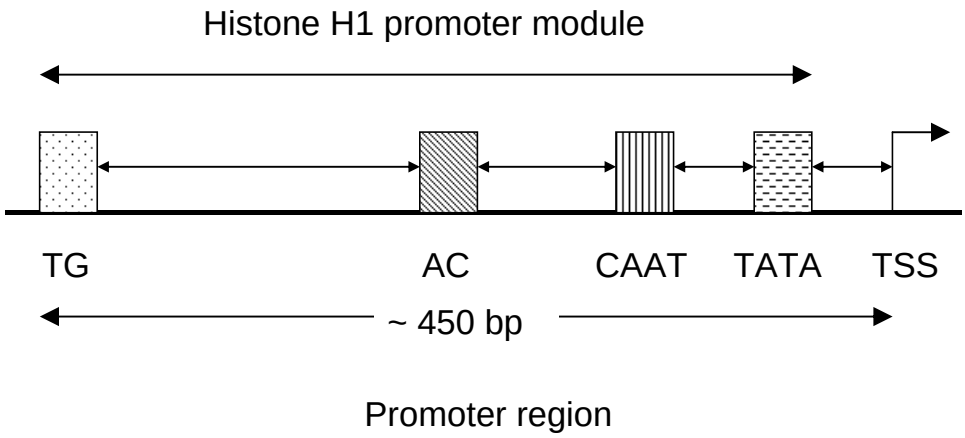


Fig 2.2: A typical promoter structure showing modular organization of TFBSs.

2.2 Why is it difficult to model promoters computationally?

The obstacles in efficient modeling and recognition of promoters are as follows:

i) promoters constitute a very small fraction of the entire genome.

ii) high variability in length of promoter; may range from a few hundred bases in some genes to thousands of bases in others.

iii) promoter sequences do not generally share common features which can be easily recognized and which can be applied universally for all types of promoter recognition.

iv) TFBSs in promoters may occur in numerous combinations and order. Apart from this, the location, the orientation, and the mutual distance between the TFBSs may also vary a lot.

v) incomplete information about TFs and TFBSs, though several thousands of them have been documented in TRANSFAC database (Matys et. al. 2003).

vi) unreliable models of TFBSs produce high number of false positives on the genome.

All these together have resulted in the inability to produce an efficient computer methodology which can be used for modeling general promoters. However, with an approach focused on modeling specific promoter subclasses some of the above problems may be diluted to some extent. This is exactly what has been followed in the present study.

2.3 Promoter modeling tools and resources.

Development of promoter modeling programs usually requires two parts, namely, the training data and a model. The model is a conceptual realization of the physical reality and is usually based on any artificial intelligence, statistical or engineering technique. It defines a scoring technique that distinguishes patterns belonging to the modeled class from other patterns. The model is usually learned from training data. Based on the scoring technique, the model searches for the desired patterns in an input sequence and reports those that have scores above a certain threshold. It is logical to think that the accuracy of the modeling depends on the quality of the training data and the model. Normally there is a trade-off between sensitivity and specificity of the prediction results; high sensitivity usually results in poor specificity and vice-versa. The parameters of the model are usually set according to one's needs.

Many of the promoter modeling programs use specialized databases for training their models. Some of these databases include: i) database on promoter sequences, e.g. EPD (Praz et. al. 2002), ii) database on TFBS and their associated TFs, e.g. TFD (Ghosh 1993), TRANSFAC Matys et. al. 2003), IMD (Chen et. al. 1995), and iii) database on TFBS modules, e.g TRANSCOMPEL (Kel-Margoulis et. al. 2002) and TRRD (Kolchanov et. al. 2002).

Promoter modeling usually involves the following aspects:

i) characterizing the structure of an already identified promoter; this involves identifying biologically significant signals in the promoter and building a model based on them;

ii) recognizing putative promoter regions from an uncharacterized genomic sequence (query data) using the model built in step 1.

TFBSs are widely used signals for promoter characterization. They can be represented in many forms, such as: i) specific binding sites, ii) consensus binding sites and iii) position weight matrix (PWM) form. Each of these has associated advantages and disadvantages, though PWM is most informative and widely accepted (Stormo 2000, Prestridge 2000).

Discovery of TFBS motifs in the promoter regions of DNA using computational tools has been an active area of research over the past few years. This usually includes approaches where: i) TFBS models are known *apriori* and ii) TFBS models are not known *apriori (*also known as *ab-initio* motif discovery*)*. Programs that have used known TFBS models for motif discovery include, Match and Patch programs of TRANSFAC package (Matys 2003), and MAST (Bailey and Gribskov 1998). However, due to lack of reliable TFBS models researchers have often resorted to *ab-initio* motif discovery methods. Programs based on *ab-initio* motif discovery have used various computational algorithms including: a) Gibbs Sampling, b) Expectation Maximization (EM), c) Global Enumeration, and d) Phylogenetic Footprinting. Programs that use EM approach are MEME (Bailey and Elkan 1994), and Dragon Motif Finder (Yang et. al. 2004); those that use Gibbs Sampling approach are AlignAce (Hughes et. al. 2000), ANN-Spec (Workman and Stormo 2000), Gibbs motif sampler (Neuwald et. al. 1995), Gibbs recursive sampler (Thompson et. al. 2003), BioProspector (Liu et. al. 2001), Co-Bind (GuhaThakurta and Stormo 2001), and MDscan

(Liu et. al. (2002); those that use Global Enumeration approach is YMF (Sinha and Tompa 2000); and those that use Phylogenetic Footprinting based methods for identifying TFBS segments in orthologous genes include techniques by Lenhard et. al. (2003), Sandelin and Wasserman (2004), Blanchette and Tompa (2002), Blanchette et. al. (2002), Blanchette and Tompa (2003), McCue et. al. (2001), McCue et. al. (2002), and Berezikov et. al. (2004).

TFBS motifs are markers for the promoter regions of the DNA, however, they are not specific to promoters alone and may occur frequently anywhere on the DNA by chance because of their short length. Individual TFBSs thus alone cannot be used to characterize promoters in a specific way. This problem can be overcome to a certain extent by considering promoter structure modeling. This methodology treats TFBSs in a promoter region as a module instead of treating them separately. This way a promoter can be characterized in a much more specific fashion. Such a methodology is in tune with the biological finding that TFBSs together constitute a cohesive functional unit. Compared to individual motif discovery, promoter structure modeling is relatively new and less studied area.

Another type of computer programs that have been introduced in the past several years aims at general promoter prediction at the genomic level. These programs differ in their objective and methods of implementation. Some programs for example, take advantage of features in the core promoter (Matis et. al. 1996, Reese 2001) while others use features in the *entire* promoter region (Prestridge 1995, Hutchinson 1996). First generation of promoter prediction software includes GRAIL (Matis et. al. 1996), NNPP (Reese 2001), PromoterScan (Prestridge 1995), Promoter 2.0 (Knudsen 1999), and PromFind (Hutchinson 1996) among others. These software programs, however, produce results that have unsatisfactorily high number of false positives (Fickett and Hatzigeorgiou 1997, Prestridge 2000). To some extent the exceptions here are GRAIL and PromoterScan, but their performance is very much hampered by the insufficiently high

sensitivity. Second generation of software produced far better results with considerably reduced level of false positives while maintaining relatively high level of sensitivity. These types of programs include PromoterInspector (Scherf et. al. 2000), Eponine (Down and Hubbard 2002), CpG-Promoter (Ioshikhes and Zhang 2000), McPromoter (Ohler et. al. 2002), FirstEF (Davuluri et. al. 2001), CpGProD (Ponger and Mouchiroud 2002), the system by Hannenhalli, Levy (Hannenhalli and Levy 2001), Dragon Promoter Finder (Bajic et. al. 2002a, 2002b, 2003), Dragon Gene Start Finder (Bajic and Seah 2003a, 2003b) and method by Narang et. al. (2005) Of these, Dragon Gene Start Finder and FirstEF show better performance based on the results on three human chromosomes (4, 21 and 22) (Bajic and Seah 2003a) as well as on the whole human genome (Bajic et. al. 2004). Apart from human, there have been other similar studies on promoters aimed at particular species, such as, fruit fly (Ohler 2006, Ohler et. al. 2002, Reese 2001, Schroeder et. al. 2004, Fiedler et. al 2006).

General promoter prediction programs do not perform well in predicting promoters of particular functional classes. This led to the development of computer programs that specifically focus upon a specific class of promoters. Such programs are based on the hypothesis that promoters of a particular functional class share common structural features. Some of these programs include the ones created for glucocorticoid and heat-shock responsive promoters (Claverie and Sauvaget 1985), globin family promoters (Staden 1988), muscle specific promoters (Wasserman and Fickett 1998, Klingenhoff et. al. 2002), liver specific promoters (Krivan and Wasserman 2001), and orthologous gene promoters (Wasserman et. al. 2000). These pioneering research efforts provided some insights into the promoter structures of specific gene families.

Many different techniques have been proposed in the past that could be used to model promoter structure of specific class of promoters, ranging from simple binary scoring schemes (Halfon et. al. 2002, Berman et. al. 2002, Markstein et. al. 2002, Frech et. al. 1997, Klingenhoff et. al. 1999,

Sosinsky et. al. 2003) to more sophisticated techniques like, logistic regression (Wasserman and Fickett 1998, Krivan and Wasserman 2001), and Hidden Markov Models (HMMs) (Grundy et. al. 1997, Frith et. al. 2001, 2002, 2003, Bailey and Noble 2003, Sinha et. al. 2003). Though most of these programs are statistical in nature, their design objectives and strategies vary. For example, for motif discovery, which forms part of promoter structure modeling, some researchers have followed IUPAC consensus (Markstein et. al. 2002) to represent TFBSs, while some others have used position weight matrices (PWMs) (Berman et. al. 2002, Frech et. al. 1997, Klingenhoff et. al. 1999, Sosinsky et. al. 2003, Grundy et. al. 1997, Frith et. al. 2002, Bailey and Noble 2003, Frith et. al. 2001, Sinha et. al. 2003). Due to their design requirements, these programs generally tend to have various built-in restrictions. For example, FastM (Klingenhoff et. al. 1999), in conjunction with ModelInspector (Frech et. al. 1997), allows generation of promoter structure models using just two TFBSs; in Cis-analyst (Berman et. al. 2002), the number of TFBS clusters to be identified within the promoter is restricted; Target Explorer (Sosinsky et. al. 2003) looks only for TFBS clusters with a fixed number of motifs specified by the user; rVISTA (Loots et. al. 2002), TraFaC (Jegga et. al. 2002), CisMols (Jegga et. al. 2005), and methods proposed by Wasserman and Fickett (1998) and by Krivan and Wasserman (2001) are based on comparative sequence analysis and thus are restricted to work only on single higher eukaryotic sequences (from one species), tending to miss species-specific TFBSs; Cis-analyst (Berman et. al. 2002), Target Explorer (Sosinsky et. al.2003), and Worm/Fly enhancer (Markstein et. al. 2002) are optimized only for the *Drosophila* genome and thus have a restrictive usage. Most of these programs consider different motif features for modelling promoter structure. For example, Target Explorer (Sosinsky et. al. 2003) and Cis-analyst (Berman et. al. 2002) consider mere presence of motifs; while Cister (Frith et. al. 2001), COMET (Frith et. al. 2002), Cluster-Buster (Frith et. al. 2003), and MCAST (Bailey and Noble 2003) take into account also the spacing between motifs; Meta-Meme (Grundy et. al. 1997) and the method proposed by Sinha et. al. (Sinha et. al. 2003) additionally considers the order of motif occurrence. Overall, these programs have their own pros

and cons when it comes to performance issues. Each one has its own limitations. Each one has its own set of parameters suitable for specific situations.

Another set of recent studies has attempted *ab-initio* modeling of promoter structure from training data (Gupta and Liu 2005, Segal and Sharan 2005). In contrast to all the studies mentioned above, the TFBSs involved in the promoters are not pre-specified in these algorithms. Only a set of related promoter sequences is provided as the input and these algorithms learn the TFBS model from the input data. These algorithms however are not designed to recognize putative promoter regions in an uncharacterized genomic sequence.

My PhD research project is an effort precisely in this direction, aimed at modeling specific class of promoter structures that belong to histone genes. The DPM system developed as a part of this research is the latest addition to the family of programs that model promoter structure. The system attempts to overcome the constraints of the abovementioned programs and has distinct advantages as shown in Chapter 5.

On the whole, there are no general solutions for promoter modeling yet. Also, for individual programs mentioned above, the detailed methodology is rarely provided, so it is not always completely clear what the model really is. Within the context of my current research I will try to provide some more general answers about a potential methodology that I have proposed for similar purposes, and I will complement this by real world examples and demonstration of its performance.

3. SPECIFIC ASPECTS RELATED TO RESEARCH PROJECT

3.1 Histone basics

Histones are basic proteins present in the eukaryotic cell nucleus. They are broadly divided into five types, namely H1, H2A, H2B, H3 and H4 (Luo and Dean 1999, Doenecke et. al. 1997). Histones range between 220 (H1) and 102 (H4) amino acids in length (Doenecke et. al. 1997) and help in packaging DNA in a highly organized structure of chromatin complex. The basic unit of this structure is the nucleosome. A nucleosome consists of about 146 bp of DNA wrapped twice around its core which is made up of two molecules each of H2A, H2B, H3, H4 (Luo and Dean 1999, Doenecke et. al. 1997). The two rounds of DNA are sealed with the nucleosome core (Luo and Dean 1999, Doenecke et. al. 1997) with the help of H1 histone, also known as linker histone. Nucleosome core, H1 histone and the linker DNA that connects two adjacent nucleosome cores, form a fundamental repeating unit of chromatin that macroscopically assumes the shape of a chromosome. Being associated with the chromosomal structure, histones play an essential role in chromosomal processes such as gene transcription, regulation, chromosome condensation, recombination and replication (Doenecke et. al. 1997). All histones, except H4, consist of several subgroups differing from each other in their primary protein structure. For example, linker histone H1 has seven subtypes named H1.1 to H1.5, H1° and H1t. Similarly, several subtypes have been reported for H2A, H2B and H3 histones (Doenecke et. al. 1997).

Based on their expression behaviour, histone genes may also be divided into three categories as: (i) S-phase of the cell cycle/DNA-replication dependent genes that are normally active during the cell proliferating stage of development such as in fetal tissues, (ii) cell-cycle independent or basally expressed replacement histone genes that tend to express in resting, differentiated cells such as in adult tissues, and (iii) tissue-specific genes that are expressed only in particular tissues

such as in germinal testis and ovary tissues. Of these three categories, a vast majority of histone genes are cell-cycle dependent genes.

Histones are evolutionarily conserved and have similar functions in all living organisms. However, the degree of conservation varies among species and within the species. Among the different histone types, the H3 and H4 histones are known to be highly conserved during evolution, while histone H1 is the least evolutionarily conserved from all histone groups (Freeman et. al. 1996, Imhof and Becker 2001). Due to the unique functions that histone proteins have in all species, it makes sense to assume that many of their genes are expressed under similar conditions. These similar conditions of co-expression are normally controlled at the main part through genes' promoters, and thus it also leads us to assume that histone promoters contain a number of common regulatory features. The present study attempts to computationally unravel such features in this important class of promoters. There has been no study in the past that analyzed a large collection of histone promoters as comprehensively as this one.

3.2 Bayesian Networks

Biological data usually have inherent inaccuracy. The inaccuracy may be due to:

i) Experimental errors

ii) Annotation errors

iii) Non-standardized experimental techniques

iv) Missing values among others, or simply

v) The nature of information contained in the data.

The present study aims at modeling promoter structure data of histone genes. Like any other biological data, the histone promoter data are also not an exception and contain inherent

inaccuracies due to reasons stated above. To model this type of data we need a computational technique that supports the uncertainty or the stochastic nature of the data. An option here is to use a technique that is based on a probabilistic modeling framework. Within this framework, I have explored Bayesian networks for the present problem, as they seem to provide a flexible and robust probabilistic modeling methodology. In principle, any AI techniques can be used for the analysis of (histone) promoter data. However, there are some inherent advantages of using Bayesian networks, which are:

i) Prior expert domain knowledge can very easily be incorporated in the model. Such knowledge is often available in biological domains.

ii) Reliable inference can be made even using small datasets.

iii) Missing values in datasets are tolerated.

iv) Both continuous and discrete variables can coexist in Bayesian networks.

v) Overfitting of data, as in maximum likelihood statistic, is avoided by the use of priors. This effectiveness means that the developed model is a better representation of the true population.

vi) Intuitive graphical representation of the problem is allowed.

vii) Causal relationships among the variables of interest can be learned using Bayesian networks. Such relationships can help gain understanding about the problem domain and can also help predict the consequences of intervention.

A Bayesian network is a model to represent and handle uncertainty in the domain knowledge. It combines probability and graph theory to explicitly represent probabilistic causal dependencies (relationships) among variables of interest in the domain knowledge (Jensen 2001). A Bayesian network has two main components:

(i) Directed acyclic graph (DAG) whose nodes represent variables and directed arrows between the nodes represent dependence relations among the variables. If there is an arc from node A to another node B, then we say that A is a parent of B. If a node in the network is known to assume a value in a hypothesis, it is said to be an evidence or observed node, else it is said to be a hidden node.

and,

(ii) A set of conditional probability distribution (CPD) for each node in the network. A CPD represents the strength of influence of the parent nodes in the network on the child nodes.

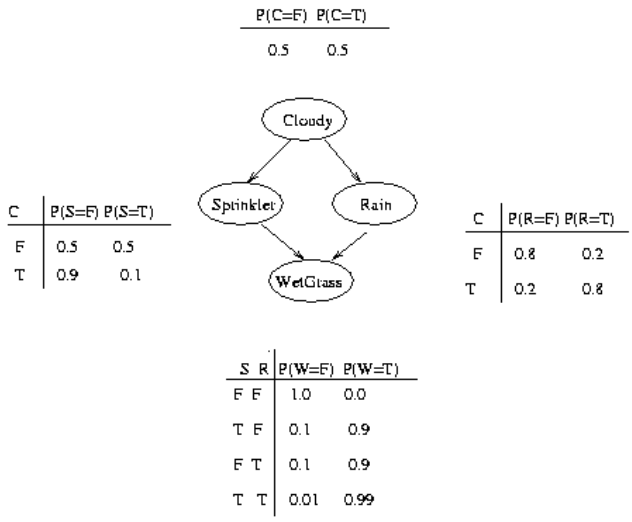


Fig 3.1 A Bayesian Network showing four nodes and their associated CPTs. *Taken from (http://www.cs.ubc.ca/~murphyk/Bayes/bayes.html)*.

A simple Bayesian network is shown in Fig 3.1. The network models an event which has four variables (nodes), namely, *Cloudy (C)*, *Sprinkler (S)*, *Rain (R)*, and *WetGrass (W)*. Each of the four nodes in the network is discrete and has two possible states/values, i.e., True=T and False=F. The arrows in the network represent the causal relationships between the nodes. For example, the states at nodes *R* and *S* influence the state of node *W*. Each of the four nodes has an associated

CPD. A CPD for a discrete node can be represented by a table, which is known as a conditional probability table (CPT). A CPT of a node contains the probability of each of the node states conditioned on the states of its parent nodes. Overall, the network represents a joint probability distribution over all its four nodes; this distribution can be viewed conceptually as a product of individual probability distributions (conditional or unconditional) at each individual node (with or without parents) (Jensen 2001). Mathematically, using the chain rule the joint probability can be written in a simplified form as,

$$P(C,\ S,\ R,\ W)\ =\ P\ (C)\ P(S\,|\,C)\ P(R\,|\,C)\ P(W\,|\,S,R) \tag{3.1}$$

where, P(C, S, R, W) is the joint probability of nodes C, S, R and W; P(C) is the marginal probability of node C; P(S|C) is the conditional probability of node S given C; P(R|C) is the conditional probability of node R given C; and P(W|S,R) is the conditional probability of node W given nodes S and R.

There are two important tasks commonly associated with Bayesian network modeling. These are i) training of model structure (DAG) and parameters (CPD), and ii) probabilistic inference using the trained model. The present study involves a pre-defined model structure and thus I would refer the term *model training* specifically for *model parameter training* in the text that follows. The training of the model is usually done by combining the training data with any prior domain knowledge that the user might have. The prior knowledge can be incorporated in the model by manipulating the arrows between the DAG nodes or by using prior probabilities in the CPD. An algorithm commonly used for training the Bayesian networks model is Expectation Maximization (EM) algorithm (Dempster et. al. 1977). A trained Bayesian model can be used for probabilistic inference. The inference basically involves calculation of probability (likelihood) of a hypothesis in the light of some evidence. This probability, also known as a degree of belief, keeps changing as the evidence accumulates. The intuition behind Bayesian inference can be explained using the

following example: consider the water sprinkler network in Fig 3.1, and suppose we observe that the grass is wet. Given this fact that the grass is wet, we would be interested in knowing which of the two causes (rain, or sprinkler on) is more likely? This question can be answered using Bayesian inference, where posterior probability is calculated for each of the above two hypotheses; the hypothesis that is more likely receives higher posterior probability. Mathematically, for example, posterior probability of the rain given that the grass is wet, can be written as,

$$P(R=T \mid W=T) = \frac{\sum_{C,S} P(C,\ S,\ R=T,\ W=T)}{P(W=T)} \tag{3.2}$$

$$P(R=T \mid W=T) = \frac{\sum_{C,S} P(C,\ S,\ R=T,\ W=T)}{\sum_{C,S,R} P(C,\ S,\ R,\ W=T)} \tag{3.3}$$

The joint probability in the above equations can be simplified by using the chain rule, as mentioned in Equation 3.1.

The general basis for Bayesian inference is the Bayes formula,

$$P(H_0 \mid E) = \frac{P(E \mid H_0)P(H_0)}{P(E)} \tag{3.4}$$

where,

H_0 represents a hypothesis.

$P(H_0)$ is the prior probability of H_0.

$P(E|H_0)$ is the conditional probability of observing the evidence E given that the hypothesis H_0 is true. It is also called the likelihood function.

P(E) is the marginal probability of *E*. It is the probability of observing the new evidence E under all mutually exclusive hypotheses. It is denoted as, $\sum_i P(E \mid H_i)P(H_i)$.

$P(H_0|E)$ is called the posterior probability of H_0 given *E*. It represents the degree of belief in the hypothesis given the evidence in the network. This is used for inference,

There are many algorithms used for solving Bayesian inference equations such as those above, however, Junction-tree algorithm (Huang and Darwiche 1994) is the most generic and widely applicable.

Bayesian networks represent an important discipline of machine learning that is widely used for making decisions in many fields. In medical field for example, a doctor might use a Bayesian network based system to diagnose his patients. By taking the observable symptoms of a patient as input, the system can predict the likelihood of the most probable disease the patient might be suffering from, and thus can assist the doctor in making a decision. Similarly, Bayesian networks have many other application areas including Bioinformatics.

4. RESEARCH PROJECT

Based on the previous overview of approaches and methods used in computational analysis of promoters, it is clear that in this domain many important problems are currently without proper solutions. The general promoter prediction will probably have to wait for some time until the high quality predictor system is developed. However, for specific classes of promoters, solutions look far closer.

Problem of function assignment to a gene based on the model of its promoter has not been solved yet. A part of this problem relates to unraveling genes that are co-regulated, because such genes are expected to have similar regulatory functions. I intend to make a contribution to this aspect of promoter analysis. The problem I want to research is related to histone promoter modeling. Although applied only to histone genes the methods to be used are of a more general nature and, in principle, could be used to model any other promoter functional groups.

4.1 Research problems

The present research project is about developing a suitable methodology for modeling histone promoters. The research problem can be divided into following parts:

i) Finding the crucial components of histone promoters.

ii) Developing a Bayesian network based classification system for modeling human histone promoters; this includes determining the optimal structure of Bayesian networks which can efficiently separate histone promoters from non-promoter DNA.

iii) Performance analysis of the developed system.

iv) Developing suitable strategy to analyze the whole human genome and search for regions that have structures similar to histone promoter model; such regions in part may represent promoters of genes that are co-regulated with histone genes.

In this research I have used the following hypothesis:

Histone genes produce evolutionarily conserved proteins with similar biological functions, thus it is reasonable to expect that these genes are co-regulated and share some common features in their promoter regions. My hypotheses for the study is that histone promoters are sufficiently homogeneous that their promoters have a lot of features in common allowing their efficient modeling by the Bayesian network approach, and that this approach allows efficient recognition of histone co-regulated genes in an anonymous DNA.

In dealing with these hypotheses I introduce the following assumptions,

- It is possible to extract sufficient number of histone genes for the intended study.
- It is possible to determine with sufficient accuracy the TSS location of the extracted histone genes.
- Modeling by Bayesian networks is a suitable technology to apply for (histone) promoter modeling.

I have conducted this research with the following delimitation in mind,

- This study does not intend to produce any commercial software based on the results of this research or in the course of research.

- This study focuses exclusively on histone promoters and efficient recognition of genes co-regulated with them.
- In the study I have exclusively used Bayesian networks for modeling and recognition of histone promoters.

4.2 Work done

This section is broadly divided into following sub-sections:

- Elucidation of histone promoter content.
- Dragon Promoter Mapper (DPM) – a promoter structure modeling system.
- Modeling of promoter structure of human histone genes using DPM.
- Comparative analysis of DPM's performance and several other systems.
- Scanning of human genome using human histone promoter structure model.

4.2.1 Elucidation of histone promoter content

In any computer modeling it is necessary to have an idea about the data. Since in my present study I endeavored to model promoter structures of histone genes, it was prudent for me to know in prior what kind of elements existed in the promoters of these genes. For this purpose, I used relevant information present in the literature and also conducted a computational analysis (Chowdhary et. al. 2005) on the histone promoter sequences.

Due to the unique functions that histone proteins have in all species, it makes sense to assume that many of their genes are expressed under similar conditions. The co-expression of histone genes implies that these genes may also be co-regulated. One of the levels at which the histone genes

are co-regulated is the transcription level (Sanchez and Marzluff, 2002; Doenecke et. al., 1994) and this suggests that their promoters may contain a number of common TFBS signals.

There have been many studies (refer reviews by, Osley 1991, Doenecke et. al. 1997) in the past that have established the presence of a number of TFBSs within the promoter regions of histone genes. Most of these studies have been experimental in nature and conducted on either single histone promoter sequence or sometimes just a handful of them. I conducted a comprehensive computational analysis on a large collection of mammalian histone promoters and confirmed the presence of several TFBS motifs shared among them. I investigated the promoter regions covering upstream [-250,-1] genomic segments relative to the TSSs in 127 histone genes from three mammalian species (human, mouse, rat). My hypothesis had been that, due to specific cellular functions complemented with a high level of protein conservation, histone genes are co-regulated and, therefore, I expected promoters of different histone groups to share common regulatory components. This study successfully elucidated the most common and significant signals present in the analyzed histone promoter sequences based on pure sequence analysis.

I was able to identify across species nine common motifs in the promoter regions of the analyzed histone genes. Table 4.1 shows the motifs that were discovered. All the motifs that I found generally corresponded well with the known TFBS in terms of composition and position. The putative binding sites represented by all the predicted motifs have been implicated in the regulation of histone genes. While CAAT-box, E2F-box, AC-box, Oct-1 binding site and H4TF2-binding site are generally known to regulate cell cycle-dependent expression of histone genes (Doenecke et. al. 1997, Oswald et. al. 1996, vanWijnen et. al. 1996), TATA-box is essential for the formation of transcription machinery (Nakajima et. al. 1988) and is found in many other genes, and GC-box is necessary for regulating many cell cycle-independent histone genes whose

expressions are widespread in many differentiated cell-lines, such behaviour is similar to housekeeping genes where GC-box is commonly found (Turner and Crossley, 1999).

Motif Number	**Motif definition**	**TFBS and associated factors**	**Transfac Site Number**
1	TCTGATTGGTTA	CCAAT-box: *H1TF2* (La Bella et. al.. 1989; Martinelli and Heintz 1994; Gallinari et. al.. 1989), *HiNF-B* (van Wijnen et. al.. 1988a,b), *NF-Y* (Mantovani 1999), *HiNF-D* (van Wijnen et. al. 1996; Grimes et. al.. 2003)	R00660
2	ATGCAAATGAGG	*Oct-1*: Octamer transcription factor 1 (*OTF-1*) (Fletcher et. al. 1987)	R00662
3	CTATAAAAACC	TATA-box: *TBP, TFIID* (Nakajima et. al.. 1988)	R00770
4	TTTTCGCGCCCA	*E2F*-binding site: *E2F-1* factor (Oswald et. al.. 1996)	R09798
5	CAATCAGGTCCG	*H4TF2/HinF-P* binding site: *H4TF2* (Pauli et. al. 1987, La Bella and Heintz 1991, Mitra et. al. 2003)	R00681
6	AACAAACACAA	AC-box: *H1TF1* (La Bella et. al. 1989), *HiNF-A* (van Wijnen et. al.. 1988b), *HiNF-D* (van Wijnen et. al. 1996; Grimes et. al. 2003)	R00658
7	CAGCCAATCAGA	CCAAT-box: *H1TF1* (La Bella et. al. 1989), *HiNF-B* (van Wijnen et. al.. 1988a,b), *NF-Y* (Mantovani 1999), *HiNF-D* (van Wijnen et. al. 1996; Grimes et. al. 2003), *H1TF2* (La Bella et. al. 1989; Martinelli and Heintz 1994; Gallinari et. al. 1989)	R00659, R00660
8	CCATTGGTTAAA	CCAAT-box: *H1TF2* (La Bella et. al. 1989; Martinelli and Heintz 1994; Gallinari et. al. 1989), *HiNF-B* (van Wijnen et. al. 1988a,b), *NF-Y* (Mantovani 1999), *HiNF-D* (van Wijnen et. al. 1996; Grimes et. al.. 2003)	R00660
9	CCCCGCCCCCCG	GC-box: *HiNF-C* (van Wijnen et. al. 1989), *Sp1* (Courey and Tjian 1988), *Sp3* (Birnbaum et. al. 1995; Hagen et. al. 1994)	R00684

Table 4.1: Relationship between detected motifs in histone promoters and biologically verified TFBS obtained from TRANSFAC database. *Taken from Chowdhary et. al. 2005*.

I observed that there are certain motifs that are specific to a particular histone group, while there are others that are shared between different histone groups. This indicates discriminatory as well as common nature of transcriptional regulatory elements of histone promoters. Shared motifs between groups suggest common regulatory mechanisms for genes sharing those motifs, while specific motifs within a group suggest specific regulatory channels that may be required for gene

transcription. I observed, for example, that Motif 5 (H4TF2-binding site/ H4-box) is highly specific to histone H4 group and is present in relatively less strength in histone H1 and has almost no presence in H2A, H2B and H3 histone groups. Further, I observed that within histone H1 group, Motif 5 is exclusively present in histone H1o subgroup. These observations are well supported by experimental studies where H4TF2-binding site is found in H4 (La Bella and Heintz 1991, Mitra et. al. 2003) and H1o (Dong et. al. 1995, Peretti and Khochbin 1997) histone genes. H4TF2-binding site in histone H1o replaces CAAT-box (Dong et. al. 1995) normally found in somatic H1 genes. Motif 2 (Oct-1 binding site) is another such motif which is group-specific, present mostly in H2A and H2B and to a lesser extent in H3 groups. This is consistent with the finding that Oct-1 element is present in histone H2A/H2B promoter (Albig et. al. 1999, Trappe et. al. 1999) and histone H3.3B promoter (Witt et. al. 1997, Frank et. al. 2003). All the remaining seven motifs (Motifs 1, 3, 4, 6, 7, 8 and 9) are found to be present in all the histone groups. However their relative presence in each group varies, refer Fig. 4.1.

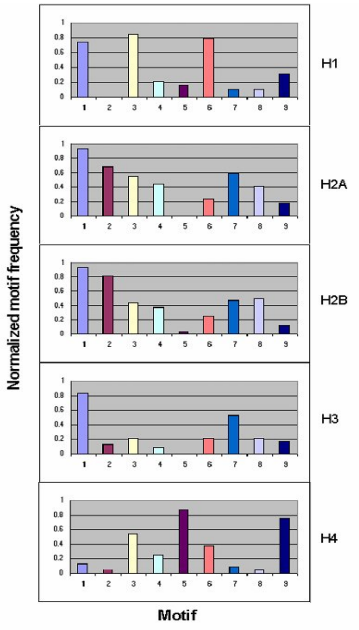


Fig. 4.1: Relative presence of motifs in different histone groups. Distribution of nine motifs found in the promoter region [-250,-1] of histone gene groups of H1, H2A, H2B, H3, and H4. Motif distribution is presented as normalized motif frequency vs. motif number (1-9). Normalized motif frequency is calculated by dividing motif frequency in a histone group by the total number of promoters in that group. *Taken from Chowdhary et. al. 2005*.

Aside motif discovery, I also analyzed the motif organization that constituted the promoter structure of these histone genes. The motif organization that I determined matched fairly well with the experimental data. For example, the consensus motif organization of histone H1 was discovered as: TATA-box, CAAT-box, GC-box, AC-box and E2F-box (order upstream of TSS with TATA-box being closest to TSS). This TFBS order is known to be specific to cell cycle-dependent H1 histone genes (Meergans et. al. 1998, Duncliffe et. al. 1995, Werner, 2001). The consensus motif organization for histone H2A and H2B groups was in accordance with previous experimental studies for somatic histone genes H2A/H2B (Oswald et. al. 1996, Albig et. al. 1999, Trappe et. al. 1999) and replacement histone genes H2A.X/H2A.Z (Yagi et. al. 1995, Oswald et. al. 1996). It was also observed that the consensus motif pattern for histone group H2A was nearly a *mirror* image of that of H2B on opposite strands. This was partly expected since the vast majority of functional H2A and H2B genes share common promoter regions on opposite strands (Albig et. al. 1999, Trappe et. al. 1999). Overall motif patterns were fairly conserved and consistent in most histone groups in terms of position, order and strand orientation.

On the whole, the motifs detected in this analysis matched fairly well with the known binding sites. Generally, the analysis succeeded in detecting over-represented TFBSs in histone promoter sequences. However, I was not able to detect all the known TFBSs in histone promoters, such as TE1 & TE2 elements in histone H1t subgroup (Grimes et. al. 2003), and RT-1 & ATF-CRE elements in H2A/H2B (Albig et. al. 1999, Trappe et. al. 1999). This may be because these binding sites were present in a small fraction of 127 histone promoters and thus probably were statistically insignificant for reporting. I also realized that as a result of the trade-off in selecting a short promoter segment [−250,−1] I was not in position to detect TFBS motifs located beyond the selected promoter region. For example, I missed motifs such as TG-box (TG-box: TGTGTTA), described first by (Duncliffe et. al. 1995) as a motif located about 450 bp upstream of the TSS in

H1 histone genes. The length of the analyzed promoter regions was purposely kept short because the extended promoter contained the genomic equivalent of the coding regions of H2A-H2B gene pairs (being bidirectional). Because of this the *ab-initio* motif detection programs, such as MEME that I used in the analysis, tend to produce too many false positives cases from the coding regions as the coding regions are generally very well conserved across histone genes.

4.2.2. Dragon Promoter Mapper [DPM] – a promoter structure modeling system

DPM is a tool to model promoter structure of co-regulated genes and has been developed as part of the present study. DPM implements a novel methodology based on Bayesian networks. DPM exploits biologically meaningful features that constitute a promoter structure, such as, motifs that represent TFBSs or any other functional or non-functional nucleotide patterns found in a promoter region. Once trained, a DPM model can be used to map (classify) a query sequence to one of the given target sequence classes (promoter and background) as defined in the training data based on the level of structure similarity between the query sequence and the target classes. In case DPM cannot map a query sequence to a target promoter class, it means that the sequence is not very similar to the target class in terms of structure. A DPM model can be used to search a genomic sequence for regions that have similar structure as the target promoter sequences. These regions may in part represent potential promoters that are co-regulated with the target promoters. The putative promoter segments detected this way may also be used as a reference for approximate assessment of their respective TSSs.

Following are the steps for using DPM:

Step 1 (Training data – refer Appendix A.1 for a sample training data file): Collect promoter sequences of transcripts assumed to be co-regulated in order to model them. Background sequences (for example random DNA sequences) may also be used. At this

stage one should know how many target sequence classes he is dealing with. For example, if a user has one promoter sequence class and one background sequence class, then the total number of target sequence classes is two.

Step 2 (Query data – refer Appendix A.1 for a sample query data file): Collect query sequences that one wants to analyze against the promoter model developed with the training data in the previous step. Query sequences may either be of the same length as the training sequences or may be *long* sequences (e.g. ~ 1000s of bp long). *Long* sequence processing details are given in Appendix A.4.

Step 3 (PWM file – refer Appendix A.1 for a sample PWM file): Find out which motifs are specific to target promoter classes in the training data. Compile a list of PWMs associated with these motifs.

Submit the training data, query data and PWM file, along with other user options to DPM. DPM builds a promoter model by using the training data, the PWM file and an automatically generated model definition file. Model definition file contains the skeleton of the Bayesian promoter model.

Step 4 (Model tuning and testing – refer Appendix A.1 for a sample model definition file): This intermediate step allows the user to modify, if necessary, the default model definition file generated by DPM. The default model in the model definition file is a Naive Bayes model (more details in section 4.2.3). DPM also provides a utility whereby one can test the performance of the model using leave-one-out cross-validation. Depending on the test results obtained, the user may wish to either proceed ahead by applying the model on the query data, or tune the model further (by modifying any or all

of these files, training data, PWM file and model definition file) and perform the test on the model again.

Step 5 (Mapping model to query data – refer Appendix A.1 for a sample output file): DPM maps the model to the query sequences. The output file contains the probability distribution for each of the query sequences over the target sequence classes defined in the model. For a *long* query sequence, the output can be used to identify the sequence regions that have similar structure as the target promoter class.

More details on the above steps can be found in the manual provided at the DPM web site (http://defiant.i2r.a-star.edu.sg/projects/BayesPromoter/html/manual/manual.htm).

DPM provides a general framework that can principally be used to model promoter structures of any category of genes. The user just needs three files (training fasta file, query fasta file and PWM file as mentioned in above steps) to run DPM in the *no-frills* mode. The *no-frills* mode, which is the default DPM setting, assumes that there are no dependencies between promoter signals (motifs, their strands, and mutual distance between adjacent motifs). Such a model represents a Naive Bayes model shown in Fig 4.4(ii) (refer http://defiant.i2r.a-star.edu.sg/projects/BayesPromoter/html/manual/Model_definition_Naive.txt for a sample Naive Bayes model file). If, however, the user is aware of any promoter signal dependencies in advance he can incorporate this biological information in his model. This can be done by modifying the *downloadable* default model definition file generated by DPM during execution time. The promoter signal dependencies are defined in the fourth block of the model definition file (refer http://research.i2r.a-star.edu.sg/DPM/Model_definition.txt for a sample file that represents model shown in Fig 4.4(iii)). Some examples of models with signal dependencies are given in Fig 4.4 where dependencies are shown sequentially between adjacent signals, however, there may be cases where additional dependencies may exist between non-adjacent signals.

DPM methodology broadly consists of two blocks, i) Bayesian model of promoter structure, and ii) Data preprocessing block. The workflow of DPM is shown in Fig 4.2.

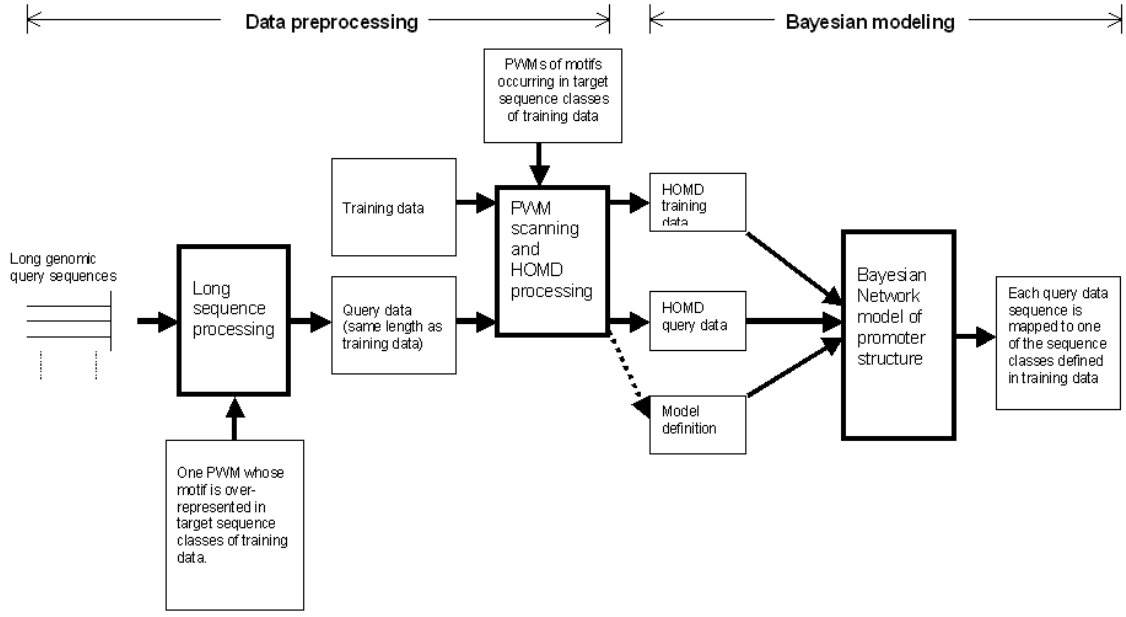


Fig 4.2: Schematic of DPM workflow. Training and query data sequences are transformed to their higher order motif definitions (HOMDs). A dotted arrow line before model definition indicates that a sample model definition file is generated by DPM with a default Naive Bayes model. *Taken from Chowdhary et. al. 2006.*

Bayesian model of promoter structure:

DPM builds a Bayesian model of promoter structure by probabilistically combining higher order features of biologically significant motifs present within the promoter sequences of interest. These features include motifs, the strand where they are found, their order of occurrence, and mutual spacer length between adjacent motifs. The nodes of the model's DAG structure encode, i) the motif features, and ii) the class of sequences used, while the arcs between these nodes encode the dependencies between them. An example of such a Bayesian promoter model is shown in Fig. 4.3 for arbitrary four motif positions. A motif position is defined as the relative

position of motif occurrence in a sequence with respect to its rightmost end (which may also be a TSS); thus the first motif that occurs in a sequence from its right end is assigned the first position, similarly the second motif is assigned the second position, the third motif is assigned the third position and so on. The number of motif positions is determined by DPM from the maximum number of motifs present in any sequence in the training data. The example model shown here has a Naive Bayes structure though DPM can principally model any structure. The Naive Bayes model shown here does not capture correlations or order between motifs. In the model, the parent node *Class* represents target sequence classes as defined in training data, and 11 child nodes represent features of each of the four motif positions occurring in a training sequence (*Mi* - motif at position *i,* and *Si* - its strand (+/-) for $i = 1, .., 4$, *i* increases away from the rightmost end of a sequence, and *L(i+1)_i* - mutual spacer length between motifs for $i = 1, .., 3$). Thus, each motif position in the training sequence points to three feature nodes of the Bayesian model, except for the first motif position which points to two feature nodes (Fig. 4.3). If no motif occurs in a training sequence for a particular motif position, the associated nodes in the model are characterized by a missing value. *Mi* and *Si* are discrete nodes, while *L(i+1)_i* is a discrete node with far too many states. In order to reduce the number of states that *L(i+1)_i* can assume, DPM discretizes *L(i+1)_i* nodes to user-defined levels. All the nodes are bound by a set of states/values they are characterized with: *Class* node, for example, may take values from target sequence classes as defined in the training data; nodes *M1* through *M4* may take values from all the names of the motifs analyzed; nodes *S1* through *S4* may take two values for plus and minus strands; and nodes *L2_1* through *L4_3* may take values corresponding to the number of states these nodes are discretized to.

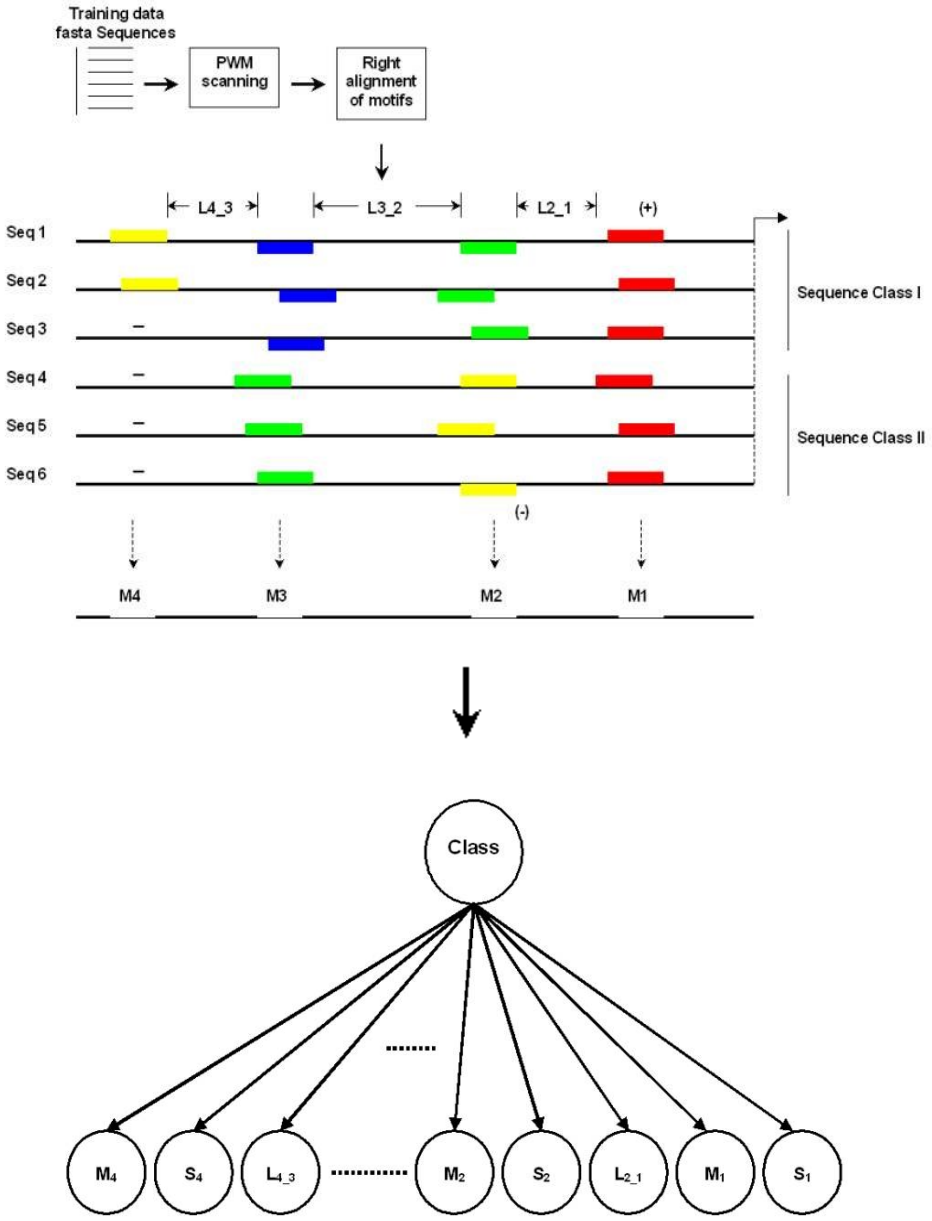


Fig 4.3: Example of a Bayesian network model of promoter structure with four motif positions. In the top panel, training sequences are shown with their higher order motif definition (HOMD). Rectangular blocks in HOMDs represent motifs, their strands are +ve if the motifs are shown above the dark horizontal line and -ve if motifs are shown below the dark horizontal line, these are also marked as (+) or (-). Thick dashes (-) represent a missing value. Lower panel shows the Bayesian model with its nodes corresponding to different higher order motif features. *Taken from Chowdhary et. al. 2006.*

DPM's Bayesian model program requires three input (intermediate) files namely, higher order motif definition (HOMD) training data, HOMD query data, and model definition (see Fig 4.2). Samples of these files are shown in Appendix A.1. HOMD training and HOMD query data contain higher order motif features of each sequence in the training and query data respectively, while the model definition contains the skeleton of the Bayesian model. Refer data preprocessing section below for details on how the HOMD files are created.

DPM uses HOMD training data and motif definition files to train the Bayesian model. DPM uses Expectation Maximization (EM) algorithm (Dempster et. al. 1977) based on uniform (Dirichlet) priors to train the model. DPM lets the user define his own model DAG structure by allowing him to manipulate the model definition file.

A trained DPM Bayesian model uses HOMD query data file for inference based on the Junction-tree algorithm (Huang and Darwiche 1994). The model returns a probability distribution for each sequence in the HOMD query data file over all the target sequence classes defined in the model. The model then classifies each HOMD query sequence to that sequence class which has the highest probability among all the target classes. Higher classification probability indicates higher similarity in sequence structures.

Data preprocessing:

The data-preprocessing block (Fig. 4.2) is basically used to convert the raw training and query fasta sequences into their HOMD formats. This is done by scanning the fasta sequences in these data sets with a set of predefined PWMs. The motif organization obtained for each sequence after PWM scanning is transformed to its HOMD format. HOMD essentially represents higher order motif features in a sequence. The data preprocessing block outputs three files that are required as input by the Bayesian model, namely, a HOMD training data, HOMD query data, and a sample

default model definition file. The inputs for the data-preprocessing block are, training data, query data, and PWM file. DPM principally can handle query sequences of any arbitrary length. However, if the query sequences are *long*, they are first processed using long sequence processing module (details of which are given in Appendix A.4).

DPM is implemented in C and PERL and uses Netica functions for Bayesian networks (Norsys Software Corp. – http://www.norsys.com). DPM webserver is available at http://defiant.i2r.a-star.edu.sg/projects/BayesPromoter.

4.2.3 Modeling of promoter structure of human histone genes using DPM

Using the methodology described in the section above, I used DPM to model promoter structure of human histone genes. Following user input files were used to build the Bayesian model:

Training Data used: Using UCSC Genome browser (http://genome.ucsc.edu) and Entrez Gene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene), I collected 68 human histone gene promoter segments covering a region of [-500,+100] with respect to the TSS. We selected a bigger promoter region in this analysis compared to our earlier study because; i) to include motifs that lie in the farther promoter regions, and ii) the PWM motif detection method we used in the present analysis was insensitive to problems (stated earlier) that the *ab-initio* method we used before was sensitive to. I also collected 10 sets of 68 non-promoter (background) sequences of the same length, selected randomly from the human genome. Thus, I had 10 sets of training data containing 136 sequences each (refer Appendix A.2 for datasets used and how they were obtained). I used multiple training sets with different background sequences in order to analyze how background sequences affected the model performance. Each sequence in the training sets belonged to either of the two classes, namely, histone promoter and non-promoter. The collected sequences corresponded to the then latest version of the human genome (HG17, May 2004).

PWMs used: As per my analysis on histone promoters I shortlisted TFBSs that were known to be present in them. Many histone promoter sequences used in the analysis were generally well annotated in the literature for the TFBSs they contained. Subsequently, based on the availability, I collected 10 PWMs corresponding to these TFBSs from different sources, such as: i) from prior biological knowledge, ii) using *ab-initio* motif discovery, iii) using TRANSFAC database (Matys et. al. 2003), and iv) using JASPAR database (Sandelin et. al. 2004). These included PWMs for TATA-box, CAAT-box, GC-box, E2F binding site, ATF/CREB binding site, Octamer1-box, AC-box, H4TF2 binding site, RT1-box, and TG-box. The PWMs so collected were then tuned on the histone promoter sequences in the training data by calculating their associated parameter/cutoff values by trial and error. The PWM file used is shown in Appendix A.1.

The training sequences (histone promoter + background) were scanned using the above-mentioned PWMs in order to obtain their HOMDs. The maximum number of motifs revealed in any training sequence after scanning was eight while the minimum number was three (refer HOMD file in Appendix A.1). The HOMD features discovered in the training sequences were used to define the nodes of the histone promoter structure Bayesian model (refer model definition file in Appendix A.1). The nodes of the model were, *M8, S8, L8_7.. M3, S3, L3_2, M2, S2, L2_1, M1, S1, Class*; these notations have been described in the above section. The possible values assumed by the nodes were: *Class: histone* and *nonPromoter*; *M:* analyzed *PWM names*; *S: plus* and *minus*; *L:* values from randomly selected 11 states (0-10 bp, 10-20 bp .. 90-100 bp, >100 bp).

In order to find a suitable DAG structure for the histone promoter structure Bayesian model, I tested arbitrarily chosen 10 DAG model structures (refer Fig. 4.4 below) in order to find the one that relatively gave the best performance. The models that I tested were based on the fact that TFBSs' order, their strands, and relative positions between adjacent TFBSs are largely conserved

and are critical to any promoter function. Thus, in the 10 histone promoter models used for analysis I used different forms of first order sequential dependencies between promoter signals. For example, model M2 represents a Naive Bayes model with no dependencies between signals; models M3, M5 and M6 capture the first order sequential dependencies between adjacent motifs and the rest represent the first order sequential dependencies between motifs and mutual lengths between them. The performance of DPM for different DAG structures was tested on the 10 training datasets mentioned above using leave-one-out cross-validation. The criteria for performance evaluation were taken as follows: if a known histone promoter sequence is classified as histone class, then it counts as a true positive (TP), else if such a sequence is classified as non-promoter class then it counts as a false negative (FN). Also, if a known background sequence is classified as non-promoter class, then it counts as a true negative (TN), else if such a sequence is classified as histone class then it counts as a false positive (FP). We define sensitivity as Se=TP/(TP+FN), and positive predictive value as ppv=TP/(TP+FP). To express overall performance quality we used correlation coefficient (cc) following (Bajic 2000),

$cc=(tp*tn-fp*fn)/[(tp+fp)(tp+fn)(tn+fp)(tn+fn)]^{0.5}$.

The DAG structures that I analyzed represented various configurations of dependence relationships between the higher order motif features. All the DAG structures used were generally based on the structure of Naive Bayes, requiring that the class node has no parent and that other nodes may or may not have the class node as their parent. Unlike Naive Bayes, however, these DAG structures allowed additional augmenting edges between attribute nodes that captured correlations among them. Each attribute node was restricted to having a maximum of one augmenting edge pointing to it. This helped controlling the computational cost (number of network parameters to be learned) in polynomial time, as shown below:

Assume,

Number of states of C (class node) = c

Maximum number of states of X_i (attribute node) = x

Number of attribute nodes = n

Number of parameters for C = c

Maximum number of parameters for an X_i which has only C as parent = c.x

Maximum number of parameters for an X_i which has C and another X_i as parent = c.x.x

Therefore, maximum total number of parameters to be learned in the Bayesian network = c + n.c.x.x. This is polynomial in variables n, c and x.

Also, complexity of EM algorithm for the network parameter learning ~ n+1 (i.e number of nodes in the network) x number of data samples for each parameter. Thus, this is also polynomial in time.

As can be seen from Fig 4.4, Naive Bayes model (model M2) has no augmenting edges. My aim was to determine which augmenting edges were most effective in improving the Naive Bayes model. Each different network configuration shown in Fig 4.4 represents some assumption about the physical interaction between binding sites, the strand on which they are located and their mutual distances. For example, addition of augmenting edges in model M3 assumes that, i) each binding site depends on preceding binding sites (either directly or indirect dependence through other binding sites), and ii) the mutual lengths between the binding sites and their strands depend on the binding sites themselves. Similarly, augmenting edges in model M1 is based on the assumption that adjacent binding sites are indirectly related through the mutual length between them.

The performance of the 10 model DAG structures, averaged over 10 training sets is shown in Table 4.2. Correlating DAG structures with their performance, it can be observed that models M3 and M4 are the same expect that in M4 direct augmenting edges between adjacent binding sites are missing and that they are indirectly connected through mutual lengths between them. Since model M3 performs better than model M4, it underlines the usefulness of direct edges between binding sites. This also supports the biological observation that mutual arrangement of binding sites in the promoter region is generally well conserved.

Comparing models M3 and M5, it can be observed that they are the same except that in model M5 node *S* is missing. Looking at the performance of M3 and M5, it appears that absence of strand in model M5 does not greatly affect the performance. Comparing models M3 and M6, it can be observed that M6 differs from M3 only in terms of *(C->L)* type of edges. The absence of these edges in M6 seems to have a marked affect on its performance (Table 4.2).

Models M4, M9 and M10 share the same network topology except that the direction of the augmenting edges *(M-S)* and *(M-L)* are different. Comparing the results of M4, M9 and M10 shows that direction of augmenting edges does not have much affect on the model performance in general. Comparing models M7 and M8 reveals that edges *(C->M)* may be important in improving the model performance.

Overall, it can be observed that model M3, which incorporates the first order direct dependence between adjacent motifs, is the best performing model on the analyzed datasets. The model definition file for model M3 is shown in Appendix A.1 (motif definition files of other analyzed models are shown in Appendix A.2). Other inferences that can be drawn from this analysis are: i) strand node *S* has an insignificant role in model performance, ii) most important edges are M->M, C->M and C->L, and iii) direction of augmenting edges may not be critically important.

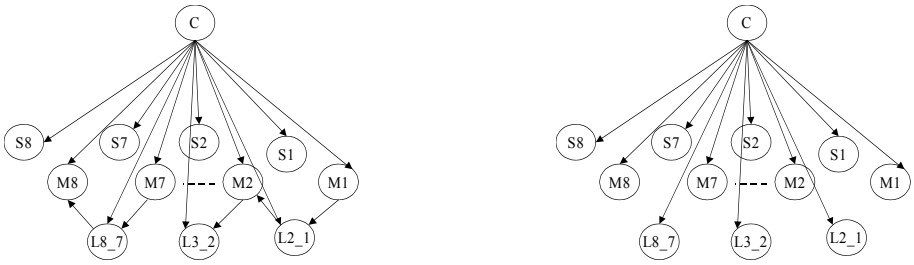
In the above analysis the model DAG structure was predefined which was partially based on what was biologically known. The model structure may also be computationally learnt from the data. However, finding a globally optimal structure from the data is NP-hard (Chickering 1994). The number of possible model structures varies super-exponentially with number of nodes in the network; for example there are $O(10^{18})$ DAGs on 10 nodes (Murphy 2001). Therefore, enumerating all possible DAGs in large networks for finding an optimal solution is not practical. Due to this researchers in the past have often used heuristics to reduce the search space. However, heuristics based algorithms, such as hill climbing, generally have the problem of converging at the local maxima. There have been recent attempts to overcome this problem by using techniques such as Simulated Annealing, multiple restarts in greedy search and Markov Chain Monte Carlo (MCMC) among others. Overall, there is no algorithm yet that can find a globally optimal model structure in a reasonable time. In order to see how well existing heuristics based structure learning algorithms work, I explored Simulated Annealing, MCMC and Greedy Search (Hill Climbing) with multiple restarts on my 10 training sets of histone promoters. For this purpose, I used the program Lib (http://www.cs.huji.ac.il/labs/compbio/LibB/programs.html) where these algorithms have been implemented. While Simulated Annealing was impractically slow, the performance of the other two algorithms averaged over the analyzed datasets is shown in Table 4.2 (detailed results of this analysis are shown in Appendix A.2). These algorithms were all run on HOMDs of the 10 training sets. The task of these algorithms was to learn Bayesian network structure of 24 nodes (1 Class node + 23 attribute nodes) from the training sets which contained the values of nodes (refer Appendix A.2 for all files used in this analysis). All the algorithms were run with default settings and with the constraint that the *Class* node was kept fixed as the root node of the network. Additionally, I also attempted to learn a Tree Augmented Naive (TAN) Bayes (Friedman et. al 1997) structure using LibB. A TAN structure has a Naive Bayes topology with optional additional augmenting edges between the attribute nodes and with a constraint that an

attribute node can have a maximum of two parent nodes including the *Class* node. However, the program apparently converged at Naive Bayes structure and did not show any correlations between attribute nodes. Overall, this analysis suggests that automated model structure learning algorithms may perhaps still be far from producing globally optimal solutions and thus may not be entirely reliable or completely match with biological findings.

Detailed results of comparative analysis of DPM histone promoter models are shown in Appendix A.2.

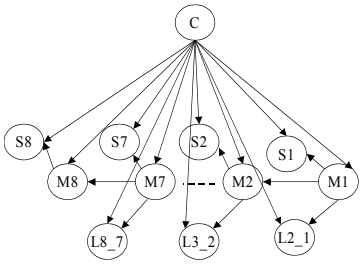
Model	Average TP	Average FP	Average Se ± stdev	Average ppv ± stdev	Average correlation coefficient (cc) ± stdev
M1	62.6	8.7	0.921 ± 0.020	0.879 ± 0.031	0.794 ± 0.048
M2	59.1	6.8	0.869 ± 0.023	0.897 ± 0.026	0.770 ± 0.043
M3	62.4	6.3	0.918 ± 0.021	0.909 ± 0.032	0.826 ± 0.039
M4	61.1	9.3	0.899 ± 0.016	0.869 ± 0.031	0.763 ± 0.038
M5	63.2	8.2	0.929 ± 0.015	0.886 ± 0.028	0.810 ± 0.041
M6	58.3	14.5	0.857 ± 0.018	0.802 ± 0.037	0.646 ± 0.057
M7	50.9	13.2	0.749 ± 0.051	0.798 ± 0.071	0.557 ± 0.108
M8	59.2	17.1	0.871 ± 0.026	0.779 ± 0.048	0.625 ± 0.075
M9	60.2	11.7	0.885 ± 0.022	0.838 ± 0.030	0.715 ± 0.044
M10	61.3	8.7	0.901 ± 0.010	0.876 ± 0.027	0.774 ± 0.033
MCMC	57.3	6.8	0.843 ± 0.035	0.894 ± 0.022	0.744 ± 0.049
Greedy Search (Hill Climbing)	57.4	6.9	0.844 ± 0.037	0.893 ± 0.022	0.744 ± 0.050

Table 4.2: Performance of 10 histone promoter structure Bayesian models (with different DAG structures) averaged over 10 analyzed datasets. Values of Se, ppv and cc are shown with their standard deviations (stdev). Also shown is the performance of automatically generated models using MCMC and Greedy search.

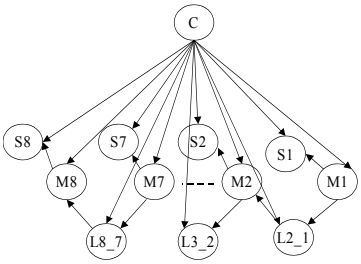


i) Model M1

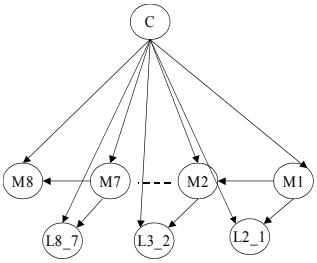
ii) Model M2



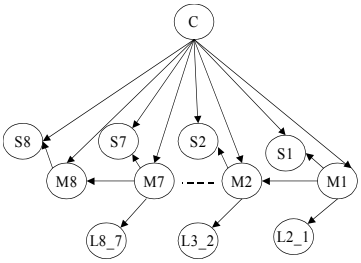
iii) Model M3



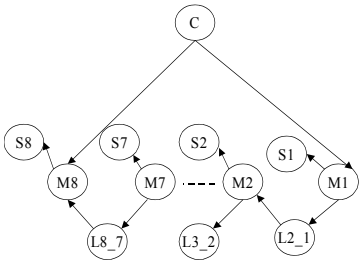
iv) Model M4



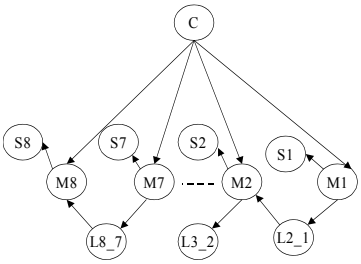
v) Model M5



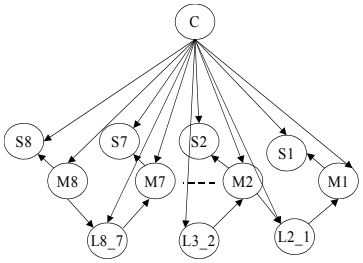
vi) Model M6



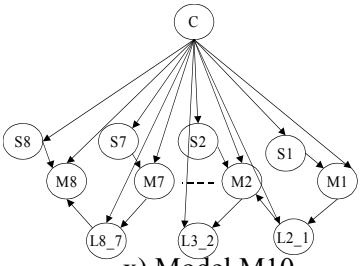
vii) Model M7



viii) Model M8



ix) Model M9



x) Model M10

Fig. 4.4: DAG structures for Bayesian networks used for modeling histone promoter.

4.2.4 Comparative analysis of DPM's performance and several other systems

In order to compare the performance of DPM with some of the well-known programs in the similar category, I chose the web servers that allowed input-data from the user in the form of test sequences and PWMs. Based on this criterion, I compared the DPM's performance with that of COMET (Frith et. al. 2002), Cluster-Buster (Frith et. al. 2003), Meta-MEME (Grundy et. al, 1997), and MCAST (Bailey and Noble 2003).

The objective of this comparative analysis was to get insight into how well the compared programs detected individual histone promoters from a test data set, as well as how well the motif distribution/arrangement within a predicted motif cluster obtained by different programs, matched with the known biological facts. It is important to note that while COMET, Cluster-Buster, Meta-MEME and MCAST report the mere presence of a cluster in a sequence, DPM additionally classifies the sequence cluster into one of the target classes.

In this comparison I applied programs in a typical scenario that a biologist will face: there are several programs available that can be used for a similar purpose to predict promoters based on the promoter region content. Also, these programs identify parts of the promoter region structures as combinations of transcription factor binding sites. However, not all programs share the same capabilities, and thus, strictly speaking, it is not possible to draw definite conclusions about programs' performances, although some aspects of the performances could be quantified. Still, we can observe in a qualitative manner the utility of DPM and compare it to the other programs.

For comparative analysis and testing of the programs, I used the data sets, PWMs, and DPM model M3, all mentioned in the previous section. The performance of DPM was analyzed on the data set using leave-one-out cross-validation. The programs COMET, Cluster-Buster, Meta-MEME and MCAST were all run with their default settings. Since COMET and Cluster-Buster

tend to combine the individual test sequences that are part of a multi-sequence file into one long sequence, I ran these programs on each test sequence separately.

The criteria for promoter recognition were: a hit was counted when a motif cluster with three or more motifs was predicted in a sequence. If, however, more than one such cluster was predicted in a sequence, they were all counted as a single hit (and not multiple hits). If a hit occurred in a promoter sequence, then such a promoter sequence was counted as a true positive, while if a hit occurred in a non-promoter (background) sequence, then such a promoter sequence was counted as a false positive.

Table 4.3 shows the results obtained by the compared programs (more detailed results of this analysis are shown in Appendix A.2). Though COMET and Cluster-Buster produced fewer false positive cases than DPM, they also predicted fewer true positive cases. Overall, DPM outperforms all other programs on the analyzed test data.

Model	Average TP	Average FP	Average Se ± stdev	Average ppv ± stdev	Average cc ± stdev
COMET	46.0	2.7	0.676 ± 0.000	0.946 ± 0.039	0.665 ± 0.037
Cluster-Buster	55.0	3.2	0.809 ± 0.000	0.946 ± 0.025	0.770 ± 0.026
Meta-Meme	67.0	42.0	0.985 ± 0.000	0.615 ± 0.022	0.460 ± 0.049
MCAST	49.0	36.3	0.721 ± 0.000	0.576 ± 0.036	0.192 ± 0.076
DPM	62.4	6.3	0.918 ± 0.021	0.909 ± 0.032	0.826 ± 0.039

Table 4.3: Performance of motif cluster finding programs averaged over 10 analyzed datasets with the minimum number of motifs in a predicted cluster kept at three. Values of Se, ppv and cc are shown with their standard deviations (stdev).

In order to assess how well individual motifs within the predicted clusters reported by the analyzed programs correspond with the known biological facts, I present motif distribution/arrangement within the clusters that were reported for five cell-cycle dependent H1

histone gene promoters (HIST1H1A, HIST1H1B, HIST1H1C, HIST1H1D, HIST1H1E). For this purpose, if a program predicted more than one cluster in a promoter, we selected the one that matched closest to what is biologically known.

Motif distribution/arrangement within the predicted clusters in the five histone promoter sequences is shown in Table 4.4. It is difficult to express the results in a quantitative manner in a simple fashion. Thus, I resort to qualitative assessment. It can be observed that the results obtained by DPM matched much closer to what has been reported earlier (Meergans et. al. 1998, Duncliffe et. al. 1995, Osley 1991, Gallinari et. al. 1989), than is the case with the other programs. DPM was able to predict most of the biologically known conserved motifs in terms of their positions and mutual order. The other programs generally were not able to detect the genuine motifs or the ordering of motifs did not match the biological facts. Overall, it can be concluded that DPM shows on this data better relative performance in terms of correct motif predictions within a cluster. Cluster-Buster is the second best program in promoter prediction. However, motif arrangement within clusters predicted by it match poorly with the known biological findings.

However, the conclusions of this analysis cannot be generalized, as the example I have used here is a specific case and thus is biased. It can happen that other programs perform better on the other data sets. The purpose of this example, however, was to demonstrate that DPM can provide in some cases more reliable information about promoter structure than the other programs. The example used represents a typical scenario that a biologist will face: several programs with different capabilities. By this example I have demonstrated that DPM is a valuable contribution to the set of available free tools for biologists to investigate regulatory regions.

Compared programs	Motif distribution/arrangement
COMET	HIST1H1A: [+AC]13[+TATA] HIST1H1B: [+AC]56[+AC]13[+TATA] HIST1H1C: [+AC]49[+AC]77[+TATA] HIST1H1D: [+AC]52[+CAAT]16[+TATA] HIST1H1E: [+AC]78[+TATA]
Cluster-Buster	HIST1H1A: [+AC]3[-GC]-8[-TATA]-9[+TATA] HIST1H1B: [+TG]-10[-AC]105[-CAAT]45[+CAAT]170[+AC]56[+AC]6[-TATA]-7[+TATA] HIST1H1C: [+TG]-10[-AC]9[+AC]122[+AC]71[-AC]52[+AC]5[+E2F]37[+AC]54[+AC]6[-TATA]-12[-TATA]-9[+TATA] HIST1H1D: [+AC]-10[-TG]51[+CAAT]9[-TATA]-7[+TATA] HIST1H1E: [+TG]-10[-AC]189[-E2F]146[+AC]55[+AC]3[-GC]-10[-TATA]-12[-TATA]-9[+TATA]
Meta-MEME	HIST1H1A: [-AC]34[+RT1]9[-E2F]53[+AC]12[+TATA]11[+Oct1]35[+TG]17[-GC] HIST1H1B: [+AC]31[+GC]10[+AC]12[+TATA] HIST1H1C: [-TG]1[+TG]8[+AC]4[-Oct1]48[-GC]44[+AC]70[-AC]51[+AC]4[+E2F]36[+AC]53[+AC]7[-TATA]2[-GC] HIST1H1D: [+TG]8[+AC]9[+Oct1]13[+TATA]38[-Oct1]121[+AC]93[+AC]51[+CAAT]15[+TATA] HIST1H1E: [+TG]188[-E2F]145[+AC]33[+GC]7[+AC]12[+TATA]
MCAST	HIST1H1A: [-AC]34[+RT1]9[-E2F]53[+AC]12[+TATA]11[+Oct1]35[+TG]17[-GC] HIST1H1B: [+AC]31[+GC]10[+AC]12[+TATA] HIST1H1C: [+AC]7[-TATA]2[-GC] HIST1H1D: [+CAAT]15[+TATA] HIST1H1E: [+AC]12[+TATA]
DPM	HIST1H1A: [-AC]104[+GC]13[+CAAT]19[+TATA]68[+TG] HIST1H1B: [+TG]348[+AC]36[+GC]3[-CAAT]9[+CAAT]19[+TATA] HIST1H1C: [+TG]349[+AC]56[+CAAT]19[+TATA] HIST1H1D: [+TG]348[+AC]58[+CAAT]19[+TATA] HIST1H1E: [+TG]348[+AC]57[+CAAT]12[-GC]3[+TATA]
Meergans et. al., (1998)	Known binding sites in H1 histone promoters: HIST1H1A: [+CAAT]19[+TATA] HIST1H1B: [+TG]364[+AC]56[+CAAT]19[+TATA] HIST1H1C: [+TG]340[+AC]58[+CAAT]19[+TATA] HIST1H1D: [+TG]372[+AC]57[+CAAT]19[+TATA] HIST1H1E: [+TG]354[+AC]58[+CAAT]19[+TATA]
Duncliff el. al., (1995)	Mutual distance between TG-box and AC-box: HIST1H1B: [+TG]359[+AC] HIST1H1D: [+TG]355[+AC]
Duncliff et. al., (1995), Osley (1991), Gallinari et. al., (1989)	General structure of H1 histone promoter, drawn from information in the reference: [TG]350[AC]34[GC]10[CAAT]19[TATA]

Table 4.4: Motif distribution/arrangement within the clusters reported by the compared programs in five histone promoter sequences (HIST1H1A, HIST1H1B, HIST1H1C, HIST1H1D, HIST1H1E). Motifs are shown along with their strands and mutual distance between them. '-' sign with the mutual distance denotes motif overlap. Motifs shown below are: TATA-box, CAAT-box, GC-box, E2F binding site, ATF/CREB binding site, Octamer1-box, AC-box, H4TF2 binding site, RT1-box, TG-box. *Taken from Chowdhary et. al. 2006*.

Performance of general promoter prediction programs on histone promoters:

In order to see how general promoter prediction programs perform on specific class of promoters, I tested two of such well-known programs on the 10 datasets mentioned in the previous section. The programs I tested were Eponine (Down and Hubbard 2002) and Dragon Promoter Finder (Bajic et. al. 2002a, 2002b, 2003). For testing, I used their webservers with the default settings. These programs were run on the forward strands of the analyzed sequences, as the annotated TSSs in these sequences were all oriented in the forward direction. The performance criteria were: a hit was counted when a TSS was predicted in a sequence. If, however, more than one TSSs were predicted in a sequence, they were all counted as a single hit. If a hit occurred in a promoter sequence, then such a promoter sequence was counted as a true positive, while if a hit occurred in a non-promoter (background) sequence, then such a promoter sequence was counted as a false positive. The results of this analysis are summarized in Table 4.5 (more detailed results of this analysis are shown in Appendix A.2).

Model	Average TP	Average FP	Average Se ± stdev	Average ppv ± stdev	Average cc ± stdev
Eponine	17.0	0.0	0.250 ± 0.000	1.000 ± 0.000	0.378 ± 0.000
Dragon Promoter Finder	36.0	1.9	0.529 ± 0.000	0.951 ± 0.033	0.560 ± 0.028

Table 4.5: Performance of general promoter prediction programs averaged over 10 analyzed datasets. Values of Se, ppv and cc are shown with their standard deviations (stdev).

Apart from these programs, I also attempted to test Dragon Gene Start Finder (Bajic and Seah 2003a, 2003b) and FirstEF (Davuluri et. al. 2001) on the histone promoter dataset. However, these programs failed to recognize a single histone promoter sequence. Dragon Gene Start Finder and FirstEF exploit junction properties between first exon and intron are thus optimized for genes that contain introns. Histone genes are mostly single exon genes and therefore Dragon Gene Start Finder and FirstEF are probably not suitable for their case. Additionally, the analyzed histone

sequences did not contain full exon segments and this may also have affected the performance of Dragon Gene Start Finder and FirstEF.

Overall, it is clear from the above analysis that the general promoter prediction programs are not entirely suitable for predicting this specific class of promoters.

4.2.5 Human genome scan using human histone promoter structure model

The aim of this experiment was to discover regions in the human genome that have structures similar to the structure of histone promoters. Such regions in the genome may, in part, represent promoters of genes that have increased likelihood to be co-regulated with some of the histone genes. It is generally expected that genes that have similar structures of regulatory regions are co-regulated in some way.

Using UCSC Genome browser, I collected 25 human chromosomal sequences corresponding to the human genome build, HG17. The chromosomes used for this experiment were, Chr1 through Chr22, ChrM, ChrX and ChrY. For this analysis, I also used histone promoter structure model M3 that gave the best overall performance as compared to the other models mentioned in the previous section. Additionally, I used 10 PWMs and associated parameters described in section 4.2.3. For training model M3, I used the dataset on which model M3 gave the best performance (i.e. training-data-1, refer model comparison analysis section in Appendix A.2).

Genome preprocessing

As a preprocessing step, using *long sequence processing* module of DPM the entire genome was scanned with the PWM of CAAT-box. This is because CAAT-box was the most frequently occurring TFBS in the training histone promoters (60 out of 68 histone promoters). Wherever CAAT-box was detected on the genome, a segment [-425,+175] with respect to the motif was

extracted for further analysis if its GC-nucleotide content was over 37% (which was the minimum value in training histone promoters). The segment coordinates were chosen based on the fact that CAAT-box usually occurs around 75bp upstream of the TSS in the promoters, which means it is located in the proximal promoter region upstream of the TATA-box (~30bp upstream of TSS) (Bucher 1990). Biologically, CAAT-box is a commonly found promoter element that controls temporal and spatial expression of the associated gene. The segments obtained after CAAT-box genome scan were all separately scanned with the PWMs of the ten analyzed histone promoter TFBSs. After PWM scanning, those segments that contained three or more motifs were short listed and fed to the DPM system as query sequences for further analysis. The DPM system applied the histone promoter model to the query sequences, and classified each query sequence to one of the two predefined classes (*histone promoter* and *non-promoter*). This way, I obtained regions on the genome that DPM predicted as histone class.

Prediction matching with RefSeq genes on the genome

Each of the genomic segment predicted by DPM as histone class was mapped back to the genome and extended by 500 bp on either side. I then checked if any gene annotation was available for this extended region using UCSC browser (RefSeq human gene data, build HG17, May 2004) and Entrez Gene. The RefSeq data contained entries for 18450 unique human genes.

It can be observed from Table 4.6 that there were 1351936 CAAT-box predictions by DPM in the initial genome scan. This large number of predictions is expected due to nonspecific model of CAAT-box, and thus many of these are likely to be nonfunctional. Of the 1351936 query segments analyzed around the CAAT-box motif, DPM qualified 504070 segments with three motifs or more. Of these segments with three or more motifs, DPM predicted 134626 as histone class. Of these, 16978 DPM predictions mapped (redundantly) with 23581 gene transcripts. Thus, the majority of the predictions fell in the intergenic regions.

The 23581 gene transcripts that were mapped by DPM predictions corresponded to 6432 known genes. There were four ways in which a prediction mapped a gene, namely, i) predicted segment covering the TSS of the gene, ii) segment covering end of the gene transcript, iii) segment located within the transcript, and iv) transcript located within the segment. Such positional bias of mapping of the predicted segments with gene transcripts is shown in Table 4.7. It is evident that most of the predictions fell within the gene loci. This suggests that there could possibly be regulatory regions within the gene loci. Some of the genes have previously been reported with similar features (Carninci et. al. Nature Genetics, 2006). In addition to this, there were 1334 unique genes (Appendix A.5) including most histone genes whose promoter regions, including the TSS, were covered by the predictions. These genes may have similar promoter structure as histone genes and therefore are expected to be co-regulated with histone genes.

Many of the DPM predictions overlapped with each other. Table 4.8 indicates the magnitude of overlap between predictions that were classified as histone class on each chromosome. Broadly, there were 134626 histone-class predictions that formed 84203 non-overlapping clusters.

Coexpression analysis

The 1334 genes whose promoters & TSSs were covered by the DPM predicted segments were further analyzed by checking if they co-expressed with the histone genes. This is because promoters are the most well annotated regulatory regions on the human genome and can generally be correlated with their associated genes' co-expression data. Also, there are no data yet available on genes co-regulated with histone genes which I could possibly have used directly to validate my results. For this co-expression analysis, I used UCSC Genome browser's Gene Sorter utility (with human GNF Gene Expression Atlas2 data, which are based on U133A and GNF1H Affymetrix chips). Gene Sorter returns a ranked list of at most 1000 genes based on the similarity

of the expression of each gene to the query gene. This way, I collected all co-expressed genes returned by Gene Sorter for each of the 68 histone genes analyzed. Gene Sorter did not have entry for three histone genes (Ids: 8338, 85235, 255626) so these were not considered, while the entry for gene with ID:83740 showed co-expression data for Gene ID:474382 (another histone gene not part of training data). The histone gene co-expression data collected from Gene Sorter is shown here (http://research.i2r.a-star.edu.sg/DPM/CAAT_Genome_Scan/Histone_coexpression.txt). The data contained expression information on 6052 unique genes.

Of 1334 genes predicted to be co-regulated (with similar promoter structure) with histone genes, 517 genes (Appendix A.5) were found to be co-expressed with the histone genes by validating their presence in the Gene Sorter histone gene co-expression data collected from Gene Sorter.

p-value analysis

Using hypergeometric distribution, I calculated p-value for the coexpression results using the formula: C(K,k)C(N-K,n-k)/C(N,n), where

N = # of cases in the total population,

n = # of cases in a selected subpopulation of N,

K = # of cases in the total population that has a specific characteristics,

k = # of cases in the subpopulation that has the specific characteristics,

C(x,y) = the number of ways to choose y items from a bag of x items, without replacement.

Applying the above to the present problem, we get,

N=18450 (Total number of unique genes known - RefSeq data)

n=1334 (Of 18450 known genes, 1334 genes have promoters similar to histone promoters)

K=6052 (Of 18450 known genes, 6052 genes coexpress with histone genes)

k=517 (Of 1334 genes that have promoters similar to histone promoters, 517 genes coexpress with histone genes).

This gives us a p-value = 1.173e-006 and corrected p-value = 0.0216 (using correction factor of 18450 for multiplicity testing), which suggests that the results obtained are statistically significant at cutoff p-value of 0.05.

Detection of histone genes

In this genome scan analysis DPM successfully identified 62 histone promoters from across the genome that contained the CAAT-box. Of these, 53 histone promoters were part of the training data, while the remaining nine were not. Thus, DPM was able to recognize a large number of training histone sequences that contained CAAT-box (53 out of 60). Interestingly, DPM was also able to detect nine histone promoters that were not part of the training data (refer result here http://research.i2r.a-star.edu.sg/DPM/CAAT_Genome_Scan/histone_gene_recognition_analysis.xls). Of the total 62 histone genes detected, all but one (GeneID: 9555) had their promoter regions along with their TSSs covered by the predictions.

Distribution of predictions on probability scale

In order to see how the frequency of all histone class DPM predictions (i.e. predictions with probability > 0.5) behaved against probability scale, I divided the prediction probability range between 0.5 and 1 into five equal bins and calculated the frequency of predictions in each bin. The same procedure was repeated separately for, i) predictions that mapped with known genes, ii) predictions in i) that covered the TSS of the genes, iii) predictions in ii) that were found to be co-expressed with histone genes. These four distributions are shown in Table 4.9. It can be observed that in all four cases highest number of predictions and the genes mapped lie above probability of 0.9. Broadly, number of predictions and mapped genes followed similar increasing trend against probability value. Of 134626 total predictions, 46093 predictions (34.2%) fell in the range above 0.9. Similarly, of 16978 predictions that mapped with known genes 5417 (31.9%) predictions fell

in the highest probability range; of 2107 predictions that mapped the TSS of the known genes 953 (45.2%) fell in the highest probability range; of 856 predictions that mapped TSS of the genes that were found to co-express with histone genes 417 (48.7%) fell in the highest probability range. Thus, we see that the percentage of the total among these four categories in the probability range > 0.9 was highest for the co-expressed gene category. This is expected as co-expressed genes are expected to be functionally related with histone genes and thus are likely to also be co-regulated and have similar promoter structures as histone promoters. Due to this promoter structure similarity, bulk of the co-expressed gene predictions is classified by DPM in the highest probability range.

A closer examination of the range above 0.9 also revealed that a large majority of predictions that mapped with histone genes were present in this range. In all, 56 histone gene promoters were detected (refer Appendix A.6 for detected genes) in this range compared to a total of 62 histone gene promoters that were predicted in the entire range between 0.5 and 1. This is expected because predictions that map with histone gene promoters should closely match with the DPM histone promoter model and thus should generally be assigned higher probabilities.

Biological-terms analysis

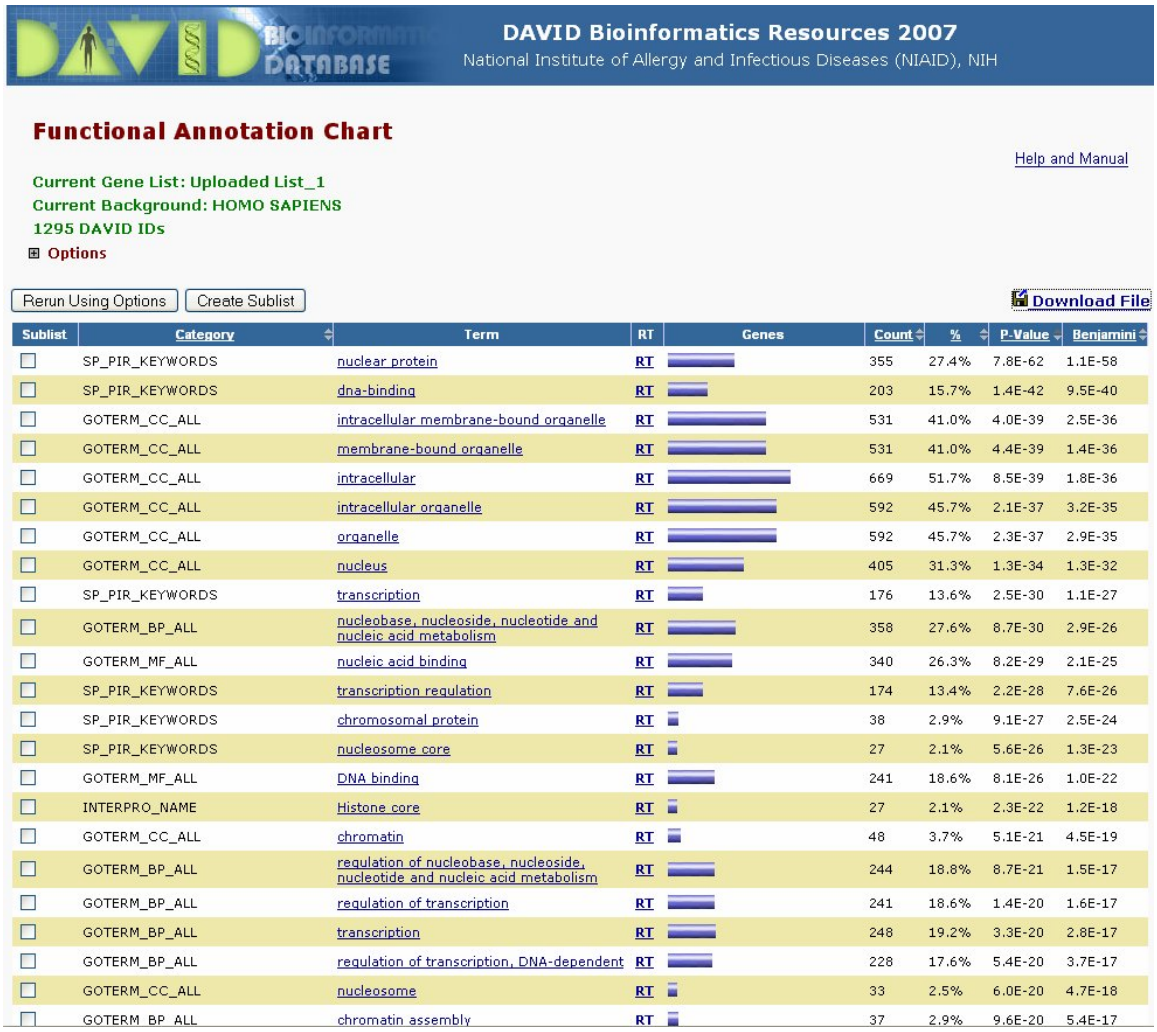
I performed functional biological annotations on the 1334 predicted histone co-regulated genes (refer Table 4.9) in order see how they were related among themselves. I used DAVID 2.1 (Dennis et. al. 2003; see also http://david.abcc.ncifcrf.gov/summary.jsp) for this purpose. Fig 4.5 is a screenshot of DAVID output which shows biologically important terms in the decreasing order of statistical significance. Below are some of the terms associated with predicted histone co-regulated genes in various functional categories (numbers in brackets represent number of genes the term is associated with):

- Top three most statistically significant terms: Nuclear protein (355), dna-binding (203) and intracellular membrane-bound organelle (531)
- Top three most frequent SP_PIR_KEYWORDS terms: nuclear protein (355), alternative splicing (218), dna-binding (203)
- Top three most frequent gene ontology (GO) molecular function (MF) terms: binding (761), nucleic acid binding (340), protein binding (332).
- Top three most frequent GO biological process (BP) terms: cellular process (814), physiological process (785), cellular physiological process (739).
- Top three most frequent GO cellular component (CC) terms: cell (788), intracellular (669) and organelle (592).

Overall, it appears that a large number of our predicted genes share many biological terms among themselves. Many of these terms are also associated with histone genes, indicating that these predicted genes with similar promoter structures may also have function related to histone genes. There appears a correlation between promoter similarity and biological terms.

No predictions on Chromosome M

From Table 4.6, it can be observed that there are no DPM predictions on Chromosome M. This suggests that mitochondrial genome does not contain any genes that are possibly co-regulated with histone genes and have similar function. This is in line with what is biologically known that mitochondrial genome is *free of histones* (Jansen 2000) and does not pack into chromatin. This is in contrast to nuclear chromosomes that pack into chromatin with the help of histones. Additionally, Chromosome M is present in the cytoplasm and not in the nucleus like nuclear chromosomes. All genes in Chromosome M are present on a single circular DNA molecule. Genes on Chromosome M are also different from genes on nuclear chromosomes in that, i) different genes on Chromosome M may share the same coding bases and ii) some codons don't



DAVID Bioinformatics Resources 2007
National Institute of Allergy and Infectious Diseases (NIAID), NIH

Functional Annotation Chart

Help and Manual

Current Gene List: Uploaded List_1
Current Background: HOMO SAPIENS
1295 DAVID IDs
Options

Rerun Using Options | Create Sublist | Download File

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
	SP_PIR_KEYWORDS	nuclear protein	RT		355	27.4%	7.8E-62	1.1E-58
	SP_PIR_KEYWORDS	dna-binding	RT		203	15.7%	1.4E-42	9.5E-40
	GOTERM_CC_ALL	intracellular membrane-bound organelle	RT		531	41.0%	4.0E-39	2.5E-36
	GOTERM_CC_ALL	membrane-bound organelle	RT		531	41.0%	4.4E-39	1.4E-36
	GOTERM_CC_ALL	intracellular	RT		669	51.7%	8.5E-39	1.8E-36
	GOTERM_CC_ALL	intracellular organelle	RT		592	45.7%	2.1E-37	3.2E-35
	GOTERM_CC_ALL	organelle	RT		592	45.7%	2.3E-37	2.9E-35
	GOTERM_CC_ALL	nucleus	RT		405	31.3%	1.3E-34	1.3E-32
	SP_PIR_KEYWORDS	transcription	RT		176	13.6%	2.5E-30	1.1E-27
	GOTERM_BP_ALL	nucleobase, nucleoside, nucleotide and nucleic acid metabolism	RT		358	27.6%	8.7E-30	2.9E-26
	GOTERM_MF_ALL	nucleic acid binding	RT		340	26.3%	8.2E-29	2.1E-25
	SP_PIR_KEYWORDS	transcription regulation	RT		174	13.4%	2.2E-28	7.6E-26
	SP_PIR_KEYWORDS	chromosomal protein	RT		38	2.9%	9.1E-27	2.5E-24
	SP_PIR_KEYWORDS	nucleosome core	RT		27	2.1%	5.6E-26	1.3E-23
	GOTERM_MF_ALL	DNA binding	RT		241	18.6%	8.1E-26	1.0E-22
	INTERPRO_NAME	Histone core	RT		27	2.1%	2.3E-22	1.2E-18
	GOTERM_CC_ALL	chromatin	RT		48	3.7%	5.1E-21	4.5E-19
	GOTERM_BP_ALL	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	RT		244	18.8%	8.7E-21	1.5E-17
	GOTERM_BP_ALL	regulation of transcription	RT		241	18.6%	1.4E-20	1.6E-17
	GOTERM_BP_ALL	transcription	RT		248	19.2%	3.3E-20	2.8E-17
	GOTERM_BP_ALL	regulation of transcription, DNA-dependent	RT		228	17.6%	5.4E-20	3.7E-17
	GOTERM_CC_ALL	nucleosome	RT		33	2.5%	6.0E-20	4.7E-18
	GOTERM_BP_ALL	chromatin assembly	RT		37	2.9%	9.6E-20	5.4E-17

Fig 4.5: Screenshot of DAVID showing biological terms shared by 1334 DPM predicted histone co-regulated genes.

follow universal translation rules. Overall, it appears that genes on Chromosome M follow different regulatory mechanism compared to nuclear genes.

This example demonstrates that DPM behaves according to our expectations, i.e. it recognizes a large proportion of the target promoter group (histones) and also recognizes other regulatory regions, including promoters, with similar structure as histone promoter model. The recognized promoters may possibly correspond to the genes that are co-regulated with histone genes. I could not validate these histone coregulated genes with any experimental data as this information is not

yet known. Instead, I validated the results with microarray based histone gene expression data on the premise that co-expressed genes are also co-regulated though this may not be true always. From the results it appears that a large number of genes are possibly co-regulated and also co-expressed with the histone genes. This may be true, as histone genes are known to have a widespread expression in tissues both in developmental and differentiated cell-lines (Doenecke et. al. 1997, Osley 1991). This widespread expression pattern of histone genes may be due to the fact that histone proteins play a critical role in chromosomal processes such as gene transcription, regulation, chromosome condensation, recombination and replication (Doenecke et. al. 1997). It generally is not possible to pass judgment for predictions that fall in the intergenic and intragenic regions. Apart from being possible false cases, these regions may in part represent regulatory regions (such as promoters, enhancers, silencers and others) associated with genes that are both known and that are possibly yet to be discovered.

Overall, this analysis has resulted in a dataset of potential genomic regulatory regions that are similar in structure to the histone promoter structure model. However, further investigations especially of experimental nature are warranted in this direction. This is precisely we plan to pursue as a next logical step by collaborating with biologists working in similar fields.

	DPM predictions			**DPM predictions mapped with annotated RefSeq genes (including histone genes)**		
Chromosome	# Predictions with CAAT-box (A)	# (A) with motifs => 3 (B)	# (B) predicted as histone class (C)	# (C) mapped with known genes	# Gene transcripts mapped with (C) (redundantly)	# Unique genes mapped with (C)
1	108973	39360	10669	1627	2220	659
2	109843	40786	10427	1190	1641	450
3	90473	34231	8642	1009	1391	372
4	78265	31004	8316	741	967	264
5	82101	31490	8179	869	1355	346
6	76965	29486	8007	1000	1249	384
7	70615	26053	6895	1011	1440	299
8	66855	25329	6543	744	953	238
9	56715	20600	5542	684	945	244
10	65527	24104	6534	956	1317	293
11	63993	23468	6037	805	1074	323
12	62499	23626	6466	800	981	348
13	39684	15488	4292	396	484	131
14	41734	15367	4001	530	757	201
15	41196	14726	4023	482	676	198
16	41963	14779	4064	641	834	245
17	39083	12970	3709	645	867	306
18	34174	12987	3431	359	472	115
19	28738	10202	3472	681	950	350
20	32508	11636	3087	420	646	147
21	15138	5803	1578	214	408	86
22	17620	5730	1743	315	521	128
M	18	5	0	0	0	0
X	74725	29971	7598	807	1344	285
Y	12531	4869	1371	52	89	20
Total	**1351936**	**504070**	**134626**	**16978**	**23581**	**6432***

Table 4.6: Human genome analysis with histone promoter model using DPM (using CAAT-box for initial scan).

* *Note that 6432 represents sum total of unique genes mapped per chromosome, while 6424 in Table 4.9 represents total unique genes mapped across the genome. The difference is because same genes sometimes are located on multiple chromosomes.*

Chromosome	Predictions upstream of transcripts (includes TSS)	Predictions downstream of transcripts	Predictions within the transcripts	Transcripts within the predictions	Total mapped transcripts
1	279	89	1829	23	2220
2	150	43	1446	2	1641
3	80	35	1276	0	1391
4	62	31	874	0	967
5	120	19	1216	0	1355
6	126	27	1032	64	1249
7	134	25	1281	0	1440
8	84	20	849	0	953
9	79	29	837	0	945
10	67	23	1227	0	1317
11	158	39	875	2	1074
12	128	33	820	0	981
13	41	6	437	0	484
14	125	17	615	0	757
15	61	15	600	0	676
16	148	37	649	0	834
17	171	31	664	1	867
18	17	7	448	0	472
19	325	100	525	0	950
20	47	22	577	0	646
21	43	7	356	2	408
22	61	21	439	0	521
M	0	0	0	0	0
X	133	45	1155	11	1344
Y	2	8	79	0	89

Table 4.7: Positional bias between predictions and gene transcript locations.

Chromosome	Total histone class predictions (redundant)	Total clusters of histone class predictions (non-redundant)
1	10669	6723
2	10427	6717
3	8642	5438
4	8316	4963
5	8179	5168
6	8007	4805
7	6895	4355
8	6543	4159
9	5542	3485
10	6534	4062
11	6037	3843
12	6466	4050
13	4292	2612
14	4001	2595
15	4023	2499
16	4064	2612
17	3709	2326
18	3431	2155
19	3472	1936
20	3087	1998
21	1578	1012
22	1743	1108
M	0	0
X	7598	4853
Y	1371	729
Total	**134626**	**84203**

Table 4.8: Overlapping/redundancy in DPM predictions that are classified as histone class.

Probability range	# DPM predictions as histone class on the entire genome (A)	# of A that mapped to known genes (B)	# of B that covered the TSS of the known genes (C)	# of C that were found to coexpress with histone genes (D)
0.90 - 1	46093	5417 (2872)	953 (613)	417 (253)
0.8 - 0.9	25144	3264 (1178)	418 (254)	154 (92)
0.7 - 0.8	21637	2742 (872)	279 (168)	110 (61)
0.6 - 0.7	19568	2589 (747)	209 (141)	95 (57)
0.5 - 0.6	22184	2966 (755)	248 (158)	80 (54)
Total	134626	16978 (6424)	2107 (1334)	856 (517)

Table 4.9: Number of DPM predictions classified as histone class in five probability bins. DPM classified a genomic segment as histone class if its prediction probability was greater than 0.5. Numbers in brackets represent unique genes mapped by the predicted genomic segments. If a single gene was mapped to multiple predictions, the gene's probability bin was considered based on the prediction with the highest probability.

5. CONCLUSION

The present study has resulted in successful development of DPM, a generic system aimed at modeling promoter structures of any class of genes. The methodology, however, can principally be applied for general purpose modeling of structures of any regulatory regions including promoters, enhancers and silencers. I have systematically illustrated the use of DPM by taking an example of an important class of genes, known as histone genes.

Compared to several similar programs, the study clearly demonstrates better performance of DPM on the analyzed datasets. Apart from this, the DPM system has several advantages compared to the existing methodologies for modeling promoter structures. These have been highlighted below along with several new concepts that have been introduced in this study:

i) first study where the promoter structure model is not rigid; a user can implement any type of correlations between motif features based on his background knowledge.

ii) first system to explicitly allow the user to test his model.

iii) first study where the methodology explicitly classifies a DNA segment with binding site clusters to one of the target classes.

iv) first study where the methodology can simultaneously handle multiple target classes of sequences.

v) first study to create an annotated data of histone promoters. To date there are only a handful of datasets known to the research community for which specific promoter models have been studied. These include the sets of i) glucocorticoid and heat-shock responsive genes (Claverie and Sauvaget 1985), ii) globin family genes (Staden 1988), iii) muscle specific genes (Wasserman and Fickett 1998, Klingenhoff et. al. 2002), and iv) liver specific genes (Krivan and Wasserman 2001). This study contributes another well-annotated dataset to the research community.

vi) first study to comprehensively model histone promoter structures computationally.

vii) first study to discover regions across the human genome that have similar structure as human histone promoters; these regions may in part represent promoters that may potentially be co-regulated with histone genes, or other regulatory regions that have similar structure as histone promoters. Such annotated data set of genomic segments can be complemented with experimental analysis, an activity that we are currently engaged with our collaborators in Germany.

I believe that research community will find DPM a useful complement to the existing set of promoter analysis tools.

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APPENDIX A:

A.1 Input and output files for the DPM system

Following are the input files required by DPM. These are either intermediate files created by the system or are user provided. The sample files shown below for training data, PWM, HOMD training data, and model definition were used in the analysis of modeling promoter structure of human histone genes.

User input files:

i) Training data: This file contains fasta DNA sequences that DPM converts to their HOMDs prior to using them for training the Bayesian model. These sequences may belong to two or more classes. For example, if there is one promoter class that a user wishes to model, then the other class may represent background (non-promoter) sequences. The class categorization and the number of classes to consider for analysis, however, depend on the modeling objectives. The sequences in the training data must all be of the same length. Note that the *Class* information should be present as the first field in the header of the fasta sequences, the format of which looks like:

```
>Class_name|any description about the sequence
actttttaaggggaaa...
Note that Class_name must not start with a numeric character.
```

Sample training data file: http://research.i2r.a-star.edu.sg/DPM/Training_data.txt

ii) Query data: This file contains fasta DNA sequences that DPM converts to their HOMDs prior predicting regions in them that match *well* with a trained Bayesian model. The query sequences may be of arbitrary length. If the query sequences are *long*, they are first processed with long sequence processing module (described below). Note that the query sequences do not contain the *Class* information in their headers.

Sample query data file: http://research.i2r.a-star.edu.sg/DPM/Query_data.txt

iii) PWM: A PWM file contains PWMs of motifs that are believed to be present in the analyzed promoter sequences. PWMs are commonly used probabilistic models for representing TFBSs. PWMs are generally obtained from resources such as, TRANSFAC, JASPAR, biological literature and ab-initio motif discovery techniques. A PWM may conceptually contain a *core region* that corresponds to the most conserved portion of the PWM. In contrast, the *matrix region* of the PWM corresponds to the entire PWM matrix. PWMs are commonly used to discover motifs in a genomic sequence. The sequence is scanned with a PWM and the motifs that meet some threshold criteria are reported back. In order to illustrate the PWM file format used by DPM, I take the example of PWM for TATA-box (taken from Bucher 1990):

```
TATA -> Name of the TFBS
Cols: 15 -> Total number of columns in the PWM to represents a TFBS
CoreStart: 2 CoreEnd: 7 -> CoreStart and CoreEnd represent the
boundaries of the core region of the PWM. The core region of the PWM
represents biologically known consensus of the TFBS and is the most
conserved part of the PWM matrix. For example, PWM core region for
TATA-box is "TATAAA" and represents columns 2 through 7 in the PWM
matrix shown below.
CoreCutoff: 0.90 MatrixCutoff: 0.85 -> CoreCutoff is the cut-off score
for the core region, while MatrixCutoff is the cut-off score for the
matrix region. Motifs with core and matrix region scores above their
respective user-defined cutoff values are considered for reporting.
These scores may range between 0 and 1.
Strand: 1 -> Which strand to scan, 1 for positive strand, while 2 for
both strands
Top: 1 -> Number of motifs desired in the output. This parameter limits
the number of motifs reported back. For example, if "Top" is set to 1,
and two motifs qualify the cutoff, then the one with the higher core
score is selected, and if the core scores are equal in both cases then
the one with the higher matrix score is selected. If for two motifs
core scores are the same, while the matrix scores are also the same,
the first identified motif is selected.
61 16 352 3 354 268 360 222 155 56 83 82 82 68 77 -> PWM
145 46 0 10 0 0 3 2 44 135 147 127 118 107 101
152 18 2 2 5 0 10 44 157 150 128 128 128 139 140
31 309 35 374 30 121 6 121 33 48 31 52 61 75 71
// -> Delimiter indicating the end of one TFBS definition.
```

Sample PWM file: http://research.i2r.a-star.edu.sg/DPM/PWM.txt

Automatically generated intermediate files:

iv) HOMD training data: This file is generated by DPM by transforming the raw training sequences to a desired HOMD format. In the file, first two lines are headers followed by the actual data, one line corresponding to one fasta sequence in the training data. The second line of the header defines the higher order motif features such as, motif name (*Mi*), strand (*Si*) and mutual spacer length between adjacent motifs (*L(i+1)_I*), for each motif position *i* = 1, 2...n, where *i* is counted from the rightmost end of a sequence, and n is the total number of motif positions. The total number of motif positions is automatically determined by DPM by counting the maximum number of motifs any sequence has in the training data. For example, in the sample HOMD training data file shown below there are eight motif positions. Missing values in the data are considered missing at random and are denoted by a "*".

Sample HOMD training data file: http://research.i2r.a-star.edu.sg/DPM/HOMD_training_data.txt

v) HOMD Query data: This file is generated by DPM by transforming raw query sequences to their desired HOMD format. The format of HOMD query data is the same as HOMD training data. Since, the class for the query sequences is unknown it is denoted by a "*" in the file.

Sample HOMD query data file: http://research.i2r.a-star.edu.sg/DPM/HOMD_query_data.txt

vi) Model definition: A model definition file basically contains the skeleton of the Bayesian network model. Each node in the Bayesian model, as defined in the model definition file, corresponds to a column in the HOMD training and HOMD query data. The number of columns in the HOMD training and HOMD query data may sometimes be more than the number of nodes defined in the model definition file. In such cases, however, columns which do not have any node entry in the model definition file are not considered in the modeling. The order of

columns in HOMD training and HOMD query data files is not important. The node/column names are case sensitive. As a utility, DPM automatically generates a sample model definition file with a default Naive Bayes model. The user may use this default model definition as a template to define his model; use the default Naive Bayes model or modify it if required. DPM also provides a leave-one-out cross validation utility for the user to test his model. DPM thus provides flexibility to the user to build and test his model before using it further. The model definition file essentially contains four blocks of parameters delimited by single blank lines (refer the sample model definition file below).

First Block: This block represents the symbols of Bayesian network nodes. Each line has a node name followed by the number of states/values the node can have. For example in the sample model definition file, the *Class* node represents the class of the sequences while 2 represents the total number of classes (refer sample HOMD training data). Similarly, the *M* nodes (*M1*, *M2* and others), *S* nodes (*S1*, *S2* and others), and *L* nodes (*L2_1*, *L3_2* and others) are presented along with the number of states/values they can assume. For example, *M* and *S* nodes which represent motif and strand, respectively, can assume 10 and 2 state values. *M* and *S* are discrete nodes. The *L* node, which represents mutual spacer length between motifs, is discretized to user-defined levels and is denoted by 0 against it.

Second Block: This block contains all state values for the discrete nodes *M, S,* and *Class*. For example, all *M* nodes may take values from the analyzed TFBS names such as, *TATA, CAAT, GC, E2F, ATFCREB, Oct1, AC, TG, H4TF2, and RT1*. The *S* nodes may take values of *plus* and *minus*. Similarly, the *Class* node can assume values as, *Histone* and *NonPromoter*. Note that node values should not start with a numeric character.

Third Block: This block is used to discretize the node L which represents the mutual spacer length between motifs. The first line represents the number of states one wants to discretize node *L* in. This is followed by the discretization levels of *L*. In the sample model definition file below, there are 12 levels that demarcate 11 states of *L*. For example 0th level is at 0, 1st level is at 10, and so on. The INFINITY_ns level represents an infinite number. If the user wishes to exclude the node (*L*) from his model, make sure to remove all the rows from the model definition file that contains *L*. Also, in such a case replace the entire third block by a 0.

Fourth block: This block defines the DAG structure of the Bayesian network model. The DAG structure gives an intuitive picture as to what dependency relationship exists between the nodes. The DAG structure shown in the sample model definition file represents a Naive Bayes model, which means that the *Class* node determines all other nodes, or all other nodes are independent of each other given the class node. Notation for example, *Class->M1*, means *Class* node determines node *M1*.

Sample model definition file: http://research.i2r.a-star.edu.sg/DPM/Model_definition.txt

Output file:

For each query sequence, DPM model outputs a probability distribution of the query sequence over all the target sequence classes. The query sequence is then assigned by the model to the target class with the highest probability. In other words this also means that of all the target sequence classes, the class that gets the highest probability is closest to the input sequence in terms of structure similarity.

Sample output file: http://research.i2r.a-star.edu.sg/DPM/DPM_output.txt

A.2 Model comparison analysis

Detailed results and datasets of comparative analysis of DPM histone promoter structure models with several other programs: http://research.i2r.a-star.edu.sg/DPM/comparison/

A.3 Files related to human genome analysis using histone promoter model

Analysis files related to genome scan using initial motif scan of CAAT-box: http://research.i2r.a-star.edu.sg/DPM/CAAT_Genome_Scan

A.4 How the *long sequence processing* module works?

This module of DPM is used when the query genomic sequence is *long* (1000s of bp long). This module first identifies the locations of the putative binding sites on the query sequence based on a single PWM selected by the user. The selected PWM may represent a biologically significant motif that is over-represented in the target promoter class. The module then extracts the segments surrounding the predicted motifs based on the user specified parameters. These parameters include, GC-cutoff for the chosen segment (maximum being 1), length of the region upstream of the motif, length of the region downstream of the motif, and minimum length between motifs (if on a strand the mutual spacer length between two detected motifs is < minimum length between motifs, the best scoring motif of them is selected for segment extraction). Minimum length between motifs can take values greater than or equal to 0; a value of -1 returns all detected motifs. It is recommended that the extracted segments should normally be of the same length as training sequences. Note that long sequence processing may sometimes generate sequences shorter than the requested range because in such cases motifs might occur near the edges of the long sequence. The sequences are scanned on both the strands and the extracted sequences are presented from 5' to 3'. Note that for PWM scanning by this module, *strand* and *top* parameters mentioned in the

PWM file are not considered. The extracted sequences are then further processed by DPM to obtain their HOMDs.

A.5 Predicted histone co-regulated/co-expressed genes.

Following 1334 Gene IDs correspond to unique genes whose TSS and promoters were covered by DPM predictions:

24 38 47 56 60 87 118 160 185 204 259 284 293 333 369 384 394 409 421 439 468 472 506 516 526 537
546 574 640 687 801 805 811 847 875 891 899 960 972 987 989 990 991 995 999 1012 1017 1028 1069
1070 1119 1149 1152 1153 1158 1161 1163 1164 1184 1280 1288 1349 1386 1408 1415 1456 1491 1503
1514 1523 1540 1605 1621 1649 1716 1717 1730 1748 1750 1785 1837 1869 1871 1912 1973 1983 1993
1994 1996 2001 2002 2010 2020 2035 2036 2068 2069 2107 2110 2118 2145 2146 2150 2185 2222 2253
2302 2316 2335 2358 2535 2569 2620 2629 2639 2651 2665 2703 2744 2752 2768 2781 2794 2804 2829
2847 2870 2879 2896 2919 2997 3006 3007 3008 3009 3010 3012 3013 3014 3017 3018 3021 3024 3028
3047 3048 3050 3104 3110 3122 3142 3146 3148 3149 3151 3178 3182 3183 3188 3190 3191 3209 3213
3239 3274 3276 3290 3304 3305 3309 3321 3350 3376 3421 3460 3516 3550 3569 3608 3642 3670 3709
3725 3726 3727 3728 3748 3775 3781 3815 3837 3840 3843 3886 3925 3930 3998 4023 4060 4074 4077
4084 4091 4108 4170 4172 4174 4176 4200 4204 4292 4329 4361 4439 4507 4548 4595 4597 4638 4643
4698 4700 4728 4735 4758 4775 4776 4793 4800 4807 4808 4809 4824 4841 4849 4856 4891 4901 4904
4925 4926 4946 4999 5007 5008 5015 5034 5037 5075 5077 5078 5080 5096 5127 5147 5165 5187 5193
5226 5241 5271 5274 5277 5290 5300 5324 5372 5383 5395 5436 5438 5454 5495 5514 5518 5525 5528
5545 5586 5686 5691 5702 5737 5757 5775 5828 5874 5902 5926 5933 5965 5971 5980 5990 5997 6009
6046 6048 6120 6142 6175 6187 6228 6238 6241 6272 6284 6299 6302 6303 6319 6349 6351 6421 6427
6428 6431 6447 6457 6503 6513 6555 6569 6605 6626 6633 6636 6651 6658 6662 6667 6722 6723 6726
6748 6776 6790 6794 6795 6811 6818 6821 6874 6895 6975 7008 7013 7025 7041 7052 7058 7071 7141
7184 7186 7259 7260 7272 7278 7289 7297 7342 7351 7353 7405 7411 7415 7453 7472 7514 7534 7536
7547 7562 7568 7589 7620 7626 7634 7639 7678 7697 7700 7726 7737 7748 7750 7763 7779 7799 7832
7846 7857 7965 8045 8106 8241 8290 8318 8320 8329 8330 8331 8332 8334 8335 8336 8337 8338 8339
8340 8341 8342 8343 8345 8346 8347 8348 8349 8350 8351 8352 8353 8355 8356 8357 8358 8359 8364
8368 8452 8467 8490 8502 8528 8650 8655 8704 8767 8804 8854 8863 8904 8943 8968 8969 8970 8975
8989 8999 9015 9019 9020 9044 9049 9055 9101 9131 9133 9146 9149 9212 9221 9230 9232 9252 9253
9325 9361 9371 9410 9464 9467 9481 9513 9521 9560 9564 9583 9601 9612 9616 9639 9645 9653 9662
9678 9682 9688 9702 9709 9715 9730 9741 9750 9751 9759 9768 9791 9793 9810 9813 9824 9855 9862
9867 9873 9886 9887 9943 9953 9993 10001 10023 10072 10092 10105 10124 10130 10131 10146 10156
10162 10163 10171 10202 10212 10214 10220 10221 10237 10238 10245 10263 10281 10289 10298
10300 10308 10311 10362 10369 10383 10420 10424 10440 10452 10459 10469 10481 10507 10525
10600 10608 10635 10658 10668 10726 10734 10738 10793 10806 10808 10810 10844 10897 10906
10912 10938 10943 10947 10956 10957 10960 10962 10963 10989 10998 11016 11068 11153 11161
11180 11182 11194 11215 11252 11259 11273 11334 11335 11339 11346 22795 22838 22847 22850
22879 22894 22897 22903 22916 22929 22933 22936 22994 23014 23030 23093 23094 23112 23130
23142 23149 23155 23193 23243 23261 23299 23301 23324 23344 23360 23397 23404 23406 23417
23462 23468 23480 23493 23523 23559 23594 23597 23635 23660 23673 23710 24138 24139 25777
25801 25822 25824 25851 25888 25901 25921 25934 25942 25994 25998 26019 26037 26064 26137
26145 26189 26261 26330 26586 26959 27072 27109 27154 27164 27235 27250 27333 27351 27434
28955 29028 29035 29086 29098 29102 29107 29123 29841 29902 29907 29944 29946 29959 29968
29985 29990 30010 30012 30819 30834 30844 49854 50512 50814 50945 51003 51050 51078 51084
51105 51114 51119 51142 51144 51150 51155 51181 51188 51203 51218 51255 51258 51259 51295
51313 51347 51361 51362 51366 51372 51412 51427 51430 51451 51514 51538 51540 51585 51603
51605 51621 51633 51741 51742 51754 53373 53916 54441 54509 54516 54537 54545 54555 54556
54567 54586 54602 54622 54677 54704 54785 54793 54820 54830 54845 54851 54868 54873 54879
54882 54897 54904 54920 54934 54935 54943 54955 54958 54962 54969 54973 54976 55007 55032

55076 55106 55124 55147 55154 55156 55159 55163 55165 55166 55253 55272 55277 55278 55282
55289 55291 55322 55329 55388 55501 55502 55510 55526 55572 55676 55702 55719 55723 55737
55751 55763 55766 55771 55776 55784 55787 55794 55821 55839 55840 55858 55889 55897 55930
55973 56001 56097 56098 56104 56105 56144 56159 56242 56267 56624 56882 56910 56922 56980
56997 57102 57149 57151 57184 57185 57464 57474 57506 57547 57575 57592 57639 57659 57693
57697 57716 57795 57799 57804 57822 58492 58515 59335 60509 60672 63922 63946 63948 64288
64344 64388 64398 64598 64710 64714 64777 64782 64795 64800 64843 64850 64975 65055 65057
65068 65083 65117 65263 65983 65988 78991 79007 79008 79009 79016 79017 79018 79019 79038
79039 79084 79086 79087 79102 79152 79165 79171 79174 79622 79624 79629 79641 79672 79682
79698 79720 79733 79744 79770 79794 79805 79848 79862 79867 79873 79877 79884 79940 79955
79973 80011 80032 80099 80185 80196 80205 80207 80217 80218 80222 80264 80274 80321 80727
80765 80772 80790 80824 81551 81558 81562 81569 81576 81610 81669 81689 81788 81850 81889
81928 81931 83401 83461 83463 83473 83592 83642 83697 83698 83740 83743 83746 84060 84172
84181 84188 84193 84206 84220 84222 84223 84229 84247 84254 84266 84268 84269 84272 84275
84279 84280 84303 84309 84312 84366 84461 84504 84527 84570 84612 84676 84681 84698 84717
84722 84734 84790 84856 84872 84876 84901 84919 84954 84964 84969 85235 85236 85316 85317
85318 85319 85416 89782 89839 89953 90139 90204 90379 90592 90861 90864 91181 91433 91543
91544 91689 91750 91942 92106 92259 92291 92591 92799 92815 92906 93058 93185 93474 93622
94039 94103 112464 112495 112714 112840 113115 113246 113451 113457 113835 114034 114043
114088 114335 114336 114789 114883 114984 115362 115509 115572 115648 115703 115827 116115
116143 116254 116328 116448 116840 117178 119391 119392 119678 120103 120237 121512 122416
122525 122773 122961 123096 124044 124411 124935 124997 125061 125113 125144 125919 125950
125965 125972 126068 126074 126231 126295 126308 126792 126961 127262 127281 127700 127833
128061 128312 129025 129531 130026 130576 130940 131578 132243 133686 134429 134492 137735
138241 139285 139562 139596 140739 142689 143684 145258 145645 146279 146330 146542 146562
147138 147183 147719 147808 147841 147965 148137 148206 148213 148254 148523 148898 149465
150274 150280 150468 151651 151871 152579 152687 153571 157313 157570 157697 158248 158947
159090 159296 161829 161835 162427 163049 163227 166012 166379 166979 167691 168374 168455
170959 170960 171392 171484 171546 195828 196294 196996 199692 199745 199777 200081 200523
200634 200844 201799 202299 202559 202865 203245 203523 205327 219541 219654 219743 219938
221443 221458 221504 221613 221656 222194 222234 252839 253260 253980 254122 254863 255403
255426 255626 255919 257068 257106 259289 259290 280658 283150 283537 283768 283991 284161
284274 284359 284390 284439 284443 284459 284525 284618 284695 285074 285172 285331 285335
285349 285605 286205 317701 317749 317772 337966 338339 338785 339175 339324 339403 339476
339487 339500 339942 340061 340252 340542 340562 340602 340665 341568 342096 343169 348235
353088 353288 373863 374393 374395 374650 375346 375513 376497 386684 387103 387882 388372
388524 388531 388815 389541 389898 390061 390535 394261 399512 399717 399833 400073 400360
400673 400932 400943 401409 401898 404734 414062 414149 425054 439940 439985 440053 440072
440073 440138 440295 440321 440686 440689 440944 441178 441242 441549 442578 442582 445329
449003 474381 474382 494115 494188 494514 548593 553115 619189 642280 643549 645078 650767
664701

Of 1334 genes above, following 517 genes were found to coexpress with histone genes:

24 56 60 118 160 204 293 369 384 468 506 516 960 987 1017 1153 1161 1163 1184 1280 1386 1415 1503
1523 1649 1730 1748 1785 1869 1871 1912 1973 1983 1994 2002 2010 2035 2222 2302 2569 2629 2639
2665 2703 2768 2794 2804 2829 2870 2997 3006 3007 3008 3009 3010 3012 3014 3017 3018 3021 3024
3028 3146 3149 3151 3178 3182 3183 3190 3213 3276 3309 3376 3421 3460 3516 3608 3670 3709 3727
3998 4170 4172 4176 4200 4292 4548 4595 4700 4728 4735 4775 4808 4809 4824 4841 4849 4901 4904
4926 4946 5007 5008 5015 5034 5078 5096 5165 5193 5277 5436 5514 5525 5528 5686 5691 5757 5828
5902 6046 6142 6175 6187 6228 6302 6303 6421 6427 6428 6431 6503 6513 6555 6626 6633 6636 6651
6723 6726 6748 6776 6794 6795 6811 6818 6874 6895 6975 7071 7289 7342 7353 7415 7453 7514 7536
7568 7589 7626 7639 7726 7737 7748 7799 7965 8045 8106 8290 8329 8330 8331 8332 8334 8335 8336
8337 8339 8340 8342 8343 8345 8346 8347 8348 8349 8350 8351 8352 8353 8355 8356 8357 8358 8359

8364 8368 8452 8968 8969 8970 8989 8999 9020 9044 9049 9131 9146 9221 9232 9252 9253 9325 9361 9371 9410 9464 9521 9583 9601 9616 9662 9682 9709 9741 9768 9791 9793 9810 9813 9855 9862 9887 9943 9953 9993 10001 10023 10092 10130 10131 10146 10156 10162 10163 10171 10212 10220 10237 10245 10263 10281 10289 10298 10362 10420 10424 10440 10452 10459 10481 10600 10608 10658 10668 10726 10738 10793 10844 10897 10943 10956 10957 10960 10989 11016 11068 11153 11180 11252 11273 22838 22847 23014 23030 23093 23130 23155 23193 23360 23480 23559 23673 25777 25801 25822 25824 25901 25921 26959 27154 27164 27333 27434 29086 29098 29102 29107 29841 29907 29946 30010 30844 50814 51003 51050 51078 51084 51142 51144 51150 51188 51347 51362 51372 51430 51540 51585 51603 51605 51741 51742 54509 54516 54545 54556 54586 54677 54785 54793 54820 54851 54868 54897 54904 54973 55007 55124 55147 55163 55253 55272 55277 55278 55289 55291 55702 55719 55751 55766 55771 55776 55784 55787 55794 55858 55930 56159 56242 56910 56922 56980 57102 57149 57151 57184 57592 57639 57693 57799 57822 60672 64598 64710 64777 64800 65083 65117 65988 79017 79039 79084 79086 79087 79171 79622 79672 79770 79794 79862 79873 80032 80099 80185 80196 80217 80218 80222 80264 81558 81562 81569 81610 81788 81931 83463 83592 83642 83743 83746 84172 84193 84247 84268 84269 84309 84461 84527 84570 84681 84717 84734 84790 84856 84901 84919 85236 85416 89782 89953 90592 91181 92259 92815 92906 93058 93622 112495 112840 113246 113451 114883 116143 116254 120103 122416 124044 125061 125919 125950 125972 126074 126231 126961 127700 128061 128312 129025 129531 131578 132243 137735 140739 145258 147808 147841 147965 148254 150274 151871 152579 153571 158947 161835 162427 166379 166979 168455 171392 171546 196294 196996 200523 202299 202865 203523 219541 219743 221613 222194 253260 257106 283150 284161 284439 286205 317701 317749 317772 338339 340061 340252 374395 376497 388524 389898 401409 474382

A.6 Histone gene prediction at probability > 0.9.

At probability > 0.9, genes with following Ids were rejected: 8341 8359 8364 9555 92815 132243. These six genes were mapped by 18 DPM predictions.

At probability > 0.9, genes with following Ids were accepted: 3006 3007 3008 3009 3010 3012 3013 3014 3017 3018 3021 3024 8290 8329 8330 8331 8332 8334 8335 8336 8337 8338 8339 8340 8342 8343 8345 8346 8347 8348 8349 8350 8351 8352 8353 8355 8356 8357 8358 8368 8968 8969 8970 55766 83740 85235 85236 126961 128312 221613 255626 317772 440686 440689 449003 474381 474382. These 57 genes were mapped by 97 DPM predictions. Thus, there was a marginal loss of 6 histone genes by increasing the cutoff from 0.5 to 0.90