

AARHUS UNIVERSITY

MASTER THESIS

Identifying signatures of human epigenetic modifications among tissues

Author:

Alejandro ROCA ARROYO

Supervisor:

Thomas BATAILLON

*A thesis submitted in fulfillment of the requirements
for the degree of Master's in Bioinformatics*

in the

Bioinformatics Research Centre BiRC

June 2, 2019

AARHUS UNIVERSITY

Abstract

Faculty of Science and Technology
Bioinformatics Research Centre

Master's in Bioinformatics

Identifying signatures of human epigenetic modifications among tissues

by Alejandro ROCA ARROYO

The number of different epigenetic landscapes for a genome may be inestimable, but we can find correlations between specific epigenetic modifications which are typically associated in concrete functions and development states. In such a way, we reduce the dimensionality of the problem making it easier to draw conclusions from the analysis of the epigenetic modifications, as well as being able to use the smaller set of correlated modifications (or signatures) as input for predictive modelling or supervised machine learning analysis.

In order to achieve this, we need to map epigenetic modification reads (from Bisulfite-seq; DNA methylations, or ChIP-seq; histone modifications) into the human genome, specifically into genes and flanking/regulatory regions, which are the ones of interest. In this way we would obtain the counts of each epigenetic modification for different tissues. Non-negative Matrix Factorization (NMF) reveals as an ideal method for the task of finding combinatorial patterns of epigenetic modifications. We can then study the state of each epigenetic modification type in the defined loci of the tissue. From this information we would obtain the different epigenetic signatures which we will use for association and simulation analysis.

Contents

Abstract	i
1 Introduction	1
1.1 Epigenetics	1
1.1.1 Epigenetic modification types	2
Histone Modifications	2
DNA methylation	2
RNA-Associated Silencing	3
Alternative Splicing	3
1.1.2 Epigenomic modeling	4
1.2 Non-negative Matrix Factorization	5
1.2.1 NMF and gene expression	6
1.2.2 NMF and mutations	6
1.2.3 NMF and epigenetics	7
2 Methods	8
2.1 NMF description	8
2.1.1 Algorithms	8
2.1.2 Choosing number of signatures	8
2.2 Bio Justification	8
2.3 Data	8
2.4 Pipeline	9
2.5 Performance	9
3 Results	10
3.1 Coverage	10
3.2 NMF Signatures	10
3.3 Association Study	10
4 Discussion	11
A Appendix A	12

List of Figures

List of Tables

List of Abbreviations

LAH List Abbreviations **Here**
WSF What (it) Stands For

Chapter 1

Introduction

1.1 Epigenetics

The genetic material is known to be modified during the life of an organism, possibly causing modifications in gene behavior. Far is known about mutations being a mayor player in genetic variation and the profiling of these variations has proved to be highly useful when studying diseases and evolutionary theory [Wray et al., 2007]. In eukaryotes, there are in addition mechanisms in which the DNA can be modified without altering the molecular sequence, so called epigenetic mechanisms. As Conrad Waddington, who coined the term “epigenetics”, defined: “it is the branch of biology which studies the causal interactions between genes and their products, which bring the phenotype into being” [Waddington, 1942]. Such definition led to categorizing as epigenetics all biological phenomena which correlated the genetic material with the genetic products and were not explained entirely by the classic genetic studies. Further studies have revealed that epigenetic mechanisms can be modulated in response to external stimuli [Liu et al., 2004, Bäckdahl et al., 2009], entailing an overlay between DNA and environment for the cells and organisms. Moreover, the epigenetic mechanisms behavior can vary for different stages of cell development [Kiefer, 2007], environmental changes [Sutherland and Costa, 2003] or disease [Jessberger et al., 2007]. In the same way genetic variability can be profiled, it is possible to decipher shared patterns for the epigenetic modifications in different scenarios and types of tissue.

Due to the latest growth on research efforts and resources about the topic, we were able to characterized the inheritance of gene expression patterns not explained by the encoded information in the DNA sequence but through epigenetic modifications. High-throughput technologies such as Chromatin Immunoprecipitation next-generation sequencing (ChIP-seq) or Whole-Genome Bisulfite sequencing (WGBS), allowed us to obtain an incredibly vast amount of information on epigenetic marks throughout the genome. It was then possible to determine the epigenome profile consisting of multiple chromatin states that activate or repress the gene expression in a local and cell-specific manner. This “epigenomic profiling” helped the understanding of cell differentiation, where even though the vast majority of cells in a multicellular organism share an identical genotype, the development of the various tissues generates stable but diverse profiles of gene expression, giving rise to the multiple cell types and differentiated cellular functions. In light of this, more specifically epigenetics may be defined as “the study of any potentially stable and, ideally, heritable change in gene expression or cellular phenotype that occurs without changes in Watson-Crick base-pairing of DNA” [Goldberg et al., 2007].

The work on nucleic acids, chromatin and histone proteins led to the understanding of the DNA arrangement, as being wrapped around the histone proteins,

and furthermore to the cytological distinction between euchromatin and heterochromatin [Elgin, 1996]. It has been proved that post-translational modifications of the histones, such as methylation, acetylation or phosphorylation, can reshape the chromatin structural and functional properties instating the concept of turning “on” and “off” regions of the genetic material. A challenging yet feasible task is to characterize those configurations responsible for the repression, activation or modulation of the gene expression via epigenetic modifications, both in different tissues and also when affected by diseases as in the case of the cancer cells used in this study. For further understanding, it is essential to know about the diverse ways epigenetic mechanisms work and elucidate the correlation among them and to biological processes.

1.1.1 Epigenetic modification types

Histone Modifications

Chromatin is conformed by the DNA molecule and a range of binding proteins into which histones are included. The DNA molecule winds around an octamer of histones, formed by dimers of four core histones: H2A, H2B, H3 and H4. Histone N-terminal tails, specially long in Histone 3 (H3), are subject of chemical modifications, such as methylation or acetylation, modulating their spatial configuration and with it, the arrangement of the chromatin. First works on the subject, in particular on histone acetylation [Schatz et al., 1964], implied a close linkage between the histone modification state and the local gene activity. This assumption was afterwards supported by experiments on histone-tail mutations in *Saccharomyces cerevisiae* [Kayne et al., 1988], hypoacetylation of the inactive X chromosome in female mammals [Jeppesen and Turner, 1993], as well as hyperacetylation of the twofold upregulated X chromosome in *D. melanogaster* males [Bone et al., 1994]. These major findings led to the compelling argument that histone modifications, along with DNA methylation, contribute to distinguish between euchromatin state and heterochromatin. Accordingly, depending on the particular histone modification profile, the chromatin can be arranged as euchromatin, which implies gene expression, or as heterochromatin, meaning the repression of the gene activity.

Chromatin state at promoters is largely invariant across diverse cell types, whereas enhancers are marked with highly cell-type-specific histone modification patterns [Heintzman and Ren, 2009]. As in methylation patterns, an aberrant histone modification pattern is associated with the development of cancer [Barneda-Zahonero and Parra, 2012]. Histone deacetylases (HDACs) are implicated expecting both positive and negative effects on oncogenic and oncosuppressive mechanisms. Again, the importance of the histone modification patterns for the gene expression and the diseases associated with an aberrant histone modification profile, call our attention on the topic.

DNA methylation

DNA methylation is perhaps the best characterized chemical modification of chromatin and were detected as early as 1948 [Hotchkiss, 1948]. In mammals, nearly all DNA methylation occurs on cytosine residues of CpG dinucleotides. Regions of the genome that have a high density of CpGs are referred to as CpG islands, and DNA methylation of these islands correlates with transcriptional expression [Deaton and Bird, 2011]. De novo or maintenance DNA methyltransferases (DNMTs) play a

critical role in gene regulation, especially those associated with transposons and imprinted genes [Goll and Bestor, 2005], by keeping the genomic patterns of cytosine methylation during embryogenesis and gametogenesis. Moreover, the formation of heterochromatin in several organisms is mediated partly by DNA methylation and its binding proteins together with RNA and histone modifications. DNA methylation takes part in many cellular processes including silencing of repetitive and centromeric sequences from fungi to mammals [Partridge et al., 2002, Jones, 2012]; X chromosome inactivation in female mammals [Weber et al., 2005]; and mammalian imprinting [Bartolomei and Ferguson-Smith, 2011], all of which can be stably maintained.

As the other epigenetic mechanisms, DNA methylation is reversible and therefore DNA methylation patterns vary in time and space during differentiation [Jones and Taylor, 1980, Xie et al., 2013]. However, abnormal control of the methylation pattern was detected in cancer cells and may result in the generation of random modification patterns which may serve to unleash new genes for transcription [Berdasco and Esteller, 2010]. The abnormal methylation pattern can be either hypomethylated, which usually involves repeated DNA sequences, or hypermethylated which involves CpG islands [Weber et al., 2005]. In the first case, oncogenes are activated whereas hypermethylation represses the transcription of the promoter regions of tumor suppressor genes, leading to gene silencing.

RNA-Associated Silencing

RNA silencing is another method to turn off genes when it is in form of antisense transcripts, noncoding RNAs or RNA interference. Antisense double-stranded RNA complementary to targeted mRNAs was detected as a method of Post-Transcriptional gene silencing (PTGS) for both cellular and viral genes in a sequence-specific manner [Hamilton and Baulcombe, 1999]. This kind of process is known as RNA-mediated interference or RNAi. Moreover, small non-coding RNAs were identified as potential 'templating' molecules for the location-specific epigenetic modifications. Several researches reported the involvement of small nuclear RNAs (snRNAs) in interacting with and presumably directing chromatin-modifying activities [Mochizuki et al., 2002, Borges and Martienssen, 2015]. The snRNAs participate in a nuclear process known as 'transcriptional gene silencing' (TGS) guiding the epigenetic machinery not only for heterochromatin assembly and gene silencing [Grewal and Moazed, 2003], but also directing programmed DNA elimination [Bernstein and Allis, 2005].

Alternative Splicing

Alternative splicing is one major mechanism that makes the most of the precursor messenger RNAs (pre-mRNAs) by processing the pre-mRNA into a diverse array of mature mRNAs that encode distinct proteins. This phenomenon explains the high complexity of organisms as humans while they have a relatively small number of protein-coding genes. Alternative splicing of RNA leads to a variety of possible mRNA isoforms and proteins, which can have different, and often opposing, functions. Sequences called exons are regions of the pre-mRNA that are included in the mature mRNA, such as the protein-coding sequences and regulatory untranslated regions at either end of the mRNA. Sequences called introns are the portions of the pre-mRNA that are removed during splicing. In alternative splicing, some sequences serve as exons under some conditions and are included in the final mRNA.

At other times, however, the alternative splicing process may exclude the same sequence, treating it as an intron and removing it from the mature mRNA.

A critical finding regarding the prevalence of alternative splicing was that a majority of human genes produce a wide variety of messenger RNAs (mRNA) that in turn encode distinct proteins [Johnson et al., 2003]. Scientists estimate that 15–60 percent of human genetic diseases involve splicing mutations, either through direct mutation of the splice-site signals or through disruption of other components of the splicing pathway [Wang and Cooper, 2007]. Therefore, understanding how the splicing machinery distinguish between exons, which are part of the mature mRNA, and introns, which are removed from the pre-mRNA, is of critical importance. Alternative splicing adds an extra layer of complexity, because regulatory sequences that sometimes designate an exon's inclusion into the mature mRNA dictate the exclusion of that exon under other conditions.

1.1.2 Epigenomic modeling

Mentioned growth on epigenetics data availability, favored by the arise of NGS sequencing techniques, granted the possibility to create extensive descriptions on the different epigenetic marks for various samples. Moreover, the findings on the function of epigenetic modifications to modulate the chromatin arrangement provided a glimpse of the likeliness to characterize the relationships between chromatin states and gene expression profiles. Thanks to the contribution made to the two major public databases, the ENCODE (The ENCYclopedia of Dna Elements) [Feingold et al., 2004] and the NIH Roadmap Epigenomics [Bernstein et al., 2010], this characterization becomes more accessible. Modeling the epigenetic modifications requires the development of computational tools which use the multiple types of data, such as DNA methylation, histone modification or Transcription Factors (TFs), in order to establish a correlation between the epigenomic assays and the biological activity. The main objectives of finding the specified models are both learn more about the biological association of epigenetic modifications and which predictions can be achieved from the models. The nature of the algorithms used to model epigenomics have been diverse but most of the proposed methods fall under unsupervised learning techniques of classification, which seek to devise patterns of chromatin modifications from the datasets alone, relying for it on multiple statistical techniques.

A first proper mathematical attempt to model histone modification dynamics was made in [Dodd et al., 2007], where they characterized the bistable gene expression resulting from the alternative states of the chromosomal regions. They applied a simple stochastic model in which they divided the DNA into 1.2 kb, corresponding to around 60 nucleosomes, and they consider either modified or unmodified nucleosomes from which they try to infer the state conversion of a region. Further contributions delved into the pattern-finding idea using more complete models such in ChromaSig [Hon et al., 2008]. Nine different types of epigenetic marks were taken into account in order to find strong signals of correlation between the epigenetic signatures profile and the promoter and enhancer activity state. Nevertheless, the analysis worked on a pre-defined set of loci with high grade of epigenetic modifications from the ENCODE project pilot, representing only the 1

More recent tools are able to directly specify the combination of epigenetic modifications or 'chromatin states' in a genome-wide fashion, making use of integrative models based on Hidden Markov Models (HMM) like in ChromHMM [Ernst and Kellis, 2010, Ernst and Kellis, 2017] and EpicSeg [Mammana and Chung, 2015], or

dynamic Bayesian networks like in Segway [Hoffman et al., 2012]. ChromHMM software learns and characterizes the chromatin states from multiple ChIP-seq datasets by creating tracks of presence/absence vectors for the epigenetic mark in a k-binned genome. In such a way, genome-wide annotation is efficient but it misses the quantitative hand of the epigenetic mark reads, using only binary data. EpicSeg uses a similar angle in the analysis, however there is no need to pre-process the data as it uses raw read counts like observations in the analysis, which allows to define a valid discrete multivariate probability distribution and then solve the loss of quantitative data. For its part, Segway refuses the idea of genome segmentation and addresses the most probable sequence of chromatin states by means of a Dynamic Bayesian Network (DBN) at a 1-bp resolution. This approach can elucidate some limitations from previous methods such as missing data handling or finding the correct constraints for segments length.

Yet, there are practical limitations essentially about the precondition to define the number of chromatin states beforehand, since this choice is arbitrary from the model and answers to biological interpretability of the results. Moreover, the algorithms mentioned above are computationally intensive and challenging to use without a sufficiently computational power. A relatively new statistical method called Non-Negative Matrix Factorization (NMF) [Lee and Seung, 1999] has been applied to the problem in question of finding chromatin states from the reads of epigenetic modification marks [Cieřlik and Bekiranov, 2014, Gandolfi and Tramontano, 2017]. NMF undertake the dimensionality problem on the genome-wide analysis of epigenetic tracks by approximating the dataset values through a reduced number of meaningful components [Devarajan, 2008]. The method makes it possible to integrate multiple epigenetic marks to the characterization of chromatin combinatory patterns, and also allows to find an optimal number of distinct patterns based on the data. In both studies [Cieřlik and Bekiranov, 2014, Gandolfi and Tramontano, 2017], they applied NMF to matrices consisting on multiple epigenetic marks as columns and non-overlapping segments of the genome as rows, being the value of the cell equal to the read counts.

In the first case, the segments used as rows correspond to multiple regions of TSS-proximal gene bodies since they contain epigenetic traces of transcription initiation and elongation. In the second case, they use bins of 200-bp, similar to the case in ChromHMM, in an attempt to approximate a single nucleosome with each bin. In the present study, aspects from these papers were used, extending the analysis to multiple cancer cell lines.

1.2 Non-negative Matrix Factorization

Non-negative matrix Factorization reveals as a method to learn parts or components from high-dimensional data. In contrast to other matrix factorization methods such as Principal Component Analysis (PCA) or Vector Quantization (VQ), NMF does not learn holistic but part-based representations. In addition, NMF is constrained to have positive values as only additive combinations are considered [Lee and Seung, 1999]. Basically, NMF consists on an approximation of an $m \times n$ V matrix by the matrix multiplication of W ($m \times k$) and H ($k \times n$). This shall be accomplished by iteratively updating the rows and columns of W and H respectively. The restriction of the data to be positive also circumscribe the use of the method, though it has been applied in astronomy [Rén et al., 2017], language processing [Bertin et al.,

2010] or image processing [Yang et al., 2007] among others. Gene expression, epigenetic modifications or mutation data have also been subject of analysis using NMF, considering the non-negativity of the data.

Dealing with high dimensionality data using NMF implies taking certain decisions, starting from the choice of NMF dimensionality (number of k signatures). An important measure used for this task is the reconstruction error, calculated by comparing the original data value with the result of the multiplication of the factorized matrices W and H . Some of the studies compare the reconstruction error produced by each defined k using the real data vs the one produced when taking random data, as it is the case in this analysis [Frigyesi and Höglund, 2008]. When examining the Residual Sum of Squares (RSS) as a function of k , the RSS decreases with increasing k within the original dataset, however, this can be less the case for the random dataset. Ideally, for a given interval of k values, there ought to be an optimal k for which the slopes of the real and random data intersect. That is to say, we intend to find the highest k , reducing the reconstruction error and improving the accuracy of the model, before being within the noise. Other studies use the cophenetic correlation coefficient, where the similarity between results from several runs is compared for each k . In other words, the stability of the classification is studied choosing the highest k before the robustness of the results drops [Brunet et al., 2004].

1.2.1 NMF and gene expression

NMF was promptly introduced in bioinformatics as a method of dimensionality reduction of large-scale gene expression data [Kim and Tidor, 2003]. Here, functional relationships are yield from the analysis of 300 genome-wide expression measurements of yeast and compared with previous expertise. The 5346 genes analyzed in 300 samples were summarized in 50 patterns from which 12 were annotated with MIPS [Mewes et al., 2002] functional categories, based on the frequency which genes from each category appeared in each of the signatures or patterns. In a similar way, NMF has also been applied to categorize tumor subtypes using 4651 human genes in 108 cases [Frigyesi and Höglund, 2008], showing that NMF signatures may correspond specific disease gene expression patterns.

1.2.2 NMF and mutations

Besides finding shared patterns between diseases and gene expression, it has been shown that it is possible to characterize specific mutations produced in varied types of cancer [Ramakrishna et al., 2012]. A catalogue of complete genomes for 21 primary breast cancer samples was sequenced and compared to the normal DNA of those same individuals. From these, 183,916 somatically acquired mutations were called and classified into 96 possible trinucleotide variations, composing the V matrix of 183916×21 cells. NMF was then applied on this dataset and from the results they identified contribution of each of the mutational signatures in each of the patients, which yield similar arrangement for similar cancer types.

Although the number of identified signatures was five, further on this number got reduced to only four signatures [Alexandrov et al., 2013]. In such a way, mutational processes operative in cancer genomes were modeled with great accuracy by a combination of this four mutational signatures. This allowed to make predictions based on the signatures and the data, as well as understand more deeply the biological processes involved on the different types of cancer.

1.2.3 NMF and epigenetics

Application of NMF to epigenetics,

Explain the use of Gandolfi, 2017 as guidance and explain differences

Gandolfi, Francesco, and Anna Tramontano. "A computational approach for the functional classification of the epigenome."

Chapter 2

Methods

2.1 NMF description

Explain the statistical framework in detail

2.1.1 Algorithms

Explain the different types of algorithms to solve the problem and the one used.

2.1.2 Choosing number of signatures

Explain different ways to choose N, the way used, the N used and why.

2.2 Bio Justification

[Change title]

Explain the relation between signatures found in NMF and biological processes

2.3 Data

The data used in this study is obtained entirely from the ENCODE project database [Feingold et al., 2004], where multiple epigenetic marks and reference epigenomes are available. Data sets from 11 types of epigenetic marks were collected, including histone modifications (H3K4me3, H3K4me1, H3K27ac, H3K36me3, H3K9me3, H3K27me3 and H3K9ac), chromatin remodeling proteins (EP300 and H2A.Z) and transcription regulation factors (CTCF, POLR2A). Replicate samples for each of the 11 epigenetic marks were used in the three cell lines of study, which includes a human liver cancer cell line (HepG2) [Aden et al., 1979], myelogenous leukemia cell line (K562) [Andersson et al., 1979] and cells derived from HeLa cancerous cervical tumor line (HeLa-S3) [Douglas, 1973, Chen, 2008].

In order to standardize the input and facilitate the data sets processing, the information for all the samples was arranged in a tab separated file with the following fields as columns:

1. **Cell line type.** Either HepG2, K562 or Hela-S3.
2. **Epigenetic modification category.** Where does the modification apply. Either histone modification ('histonemod'), chromatin modulation ('openchromatin') or transcription factor ('TFBS').

3. **Epigenetic modification name.** The label of the mark which the sample corresponds to, as mentioned above.
4. **Accession ID.** The accession name for the sample in the database.
5. **File name.**
6. **Processing status.** Either 'raw' or 'process'. Raw samples need to be pre-processed.
7. **Replicate number.** Integer enumerating the various replicates for a particular epigenetic mark.
8. **Database name.** In the present case, 'ENCODE'.
9. **Download link.** Used to download the sample reads file.

As a matter of convenience, 'downloadData.sh [DataInfo.tsv]' or 'epigeNMF.sh -d [DataInfo.tsv]' can be used to automatically download the data sets into the appropriate directory tree: CELL_LINE/SIGNAL_TRACK/SAMPLE_ID.

2.4 Pipeline

Explain the command line tool, the pipeline followed for the analysis and explain functioning of each script

You should follow the next pipeline for the analysis:

1. Download the data (option -d or --download)
2. Prepare BED alignment files (option -p or --process)
3. Create V matrix of counts for marks (cols) by bins (rows) (option -c or --counts)
4. Filter V matrix bins to remove noise (option -f or --filter)
5. Choose the optimal 'n' number of signatures for NMF (option -k or --chooseN)
6. NMF analysis (option -n or --nmf)

2.5 Performance

Perform analysis with percentage of the data and get performance (time) plots

Chapter 3

Results

3.1 Coverage

Study the epigenetic marks along the different chromosomes

3.2 NMF Signatures

H matrix

Study the NMF signatures obtained and compare between different tissues. Relate to biology.

3.3 Association Study

[MAYBE]

W Matrix

Get hotspots and study association and relation to biology.

Chapter 4

Discussion

Comparison

Convenience

Outcome

Future perspectives

Appendix A

Appendix A

Bibliography

- [Aden et al., 1979] Aden, D. P., Fogel, A., Plotkin, S., Damjanov, I., and Knowles, B. B. (1979). Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line.
- [Alexandrov et al., 2013] Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Campbell, P. J., and Stratton, M. R. (2013). Deciphering Signatures of Mutational Processes Operative in Human Cancer. *Cell Reports*.
- [Andersson et al., 1979] Andersson, L. C., Nilsson, K., and Gahmberg, C. G. (1979). K562—A human erythroleukemic cell line. *International Journal of Cancer*.
- [Bäckdahl et al., 2009] Bäckdahl, L., Bushell, A., and Beck, S. (2009). Inflammatory signalling as mediator of epigenetic modulation in tissue-specific chronic inflammation.
- [Barneda-Zahonero and Parra, 2012] Barneda-Zahonero, B. and Parra, M. (2012). Histone deacetylases and cancer.
- [Bartolomei and Ferguson-Smith, 2011] Bartolomei, M. S. and Ferguson-Smith, A. C. (2011). Mammalian genomic imprinting. *Cold Spring Harbor Perspectives in Biology*.
- [Berdasco and Esteller, 2010] Berdasco, M. and Esteller, M. (2010). Aberrant Epigenetic Landscape in Cancer: How Cellular Identity Goes Awry.
- [Bernstein et al., 2010] Bernstein, B. E., Stamatoyannopoulos, J. A., Costello, J. F., Ren, B., Milosavljevic, A., Meissner, A., Kellis, M., Marra, M. A., Beaudet, A. L., Ecker, J. R., Farnham, P. J., Hirst, M., Lander, E. S., Mikkelsen, T. S., and Thomson, J. A. (2010). The NIH Roadmap Epigenomics Mapping Consortium. *Nature biotechnology*.
- [Bernstein and Allis, 2005] Bernstein, E. and Allis, C. D. (2005). RNA meets chromatin.
- [Bertin et al., 2010] Bertin, N., Badeau, R., and Vincent, E. (2010). Enforcing harmonicity and smoothness in bayesian non-negative matrix factorization applied to polyphonic music transcription. *IEEE Transactions on Audio, Speech and Language Processing*.
- [Bone et al., 1994] Bone, J. R., Lavender, J., Richman, R., Palmer, M. J., Turner, B. M., and Kuroda, M. I. (1994). Acetylated histone H4 on the male X chromosome is associated with dosage compensation in *Drosophila*. *Genes and Development*.
- [Borges and Martienssen, 2015] Borges, F. and Martienssen, R. A. (2015). The expanding world of small RNAs in plants.

- [Brunet et al., 2004] Brunet, J.-P., Tamayo, P., Golub, T. R., and Mesirov, J. P. (2004). Metagenes and molecular pattern discovery using matrix factorization. *Proceedings of the National Academy of Sciences of the United States of America*.
- [Chen, 2008] Chen, T. (2008). Re-evaluation of HeLa, HeLa S3, and HEp-2 karyotypes. *Cytogenetic and Genome Research*.
- [Cieřlik and Bekiranov, 2014] Cieřlik, M. and Bekiranov, S. (2014). Combinatorial epigenetic patterns as quantitative predictors of chromatin biology. *BMC Genomics*, 15(1).
- [Deaton and Bird, 2011] Deaton, A. M. and Bird, A. (2011). CpG islands and the regulation of transcription. *Genes and Development*.
- [Devarajan, 2008] Devarajan, K. (2008). Nonnegative matrix factorization: An analytical and interpretive tool in computational biology.
- [Dodd et al., 2007] Dodd, I. B., Micheelsen, M. A., Sneppen, K., and Thon, G. (2007). Theoretical Analysis of Epigenetic Cell Memory by Nucleosome Modification. *Cell*.
- [Douglas, 1973] Douglas, J. (1973). HeLa. *Nature*.
- [Elgin, 1996] Elgin, S. C. (1996). Heterochromatin and gene regulation in *Drosophila*. *Current Opinion in Genetics and Development*.
- [Ernst and Kellis, 2010] Ernst, J. and Kellis, M. (2010). Discovery and characterization of chromatin states for systematic annotation of the human genome . Overview of Supplementary Materials : Supplementary Notes. *Nature Biotechnology*.
- [Ernst and Kellis, 2017] Ernst, J. and Kellis, M. (2017). Chromatin-state discovery and genome annotation with ChromHMM. *Nature Protocols*.
- [Feingold et al., 2004] Feingold, E. A., Good, P. J., Guyer, M. S., and et al. (2004). The ENCODE (ENCyclopedia of DNA Elements) Project.
- [Frigyesi and Höglund, 2008] Frigyesi, A. and Höglund, M. (2008). Non-negative matrix factorization for the analysis of complex gene expression data: identification of clinically relevant tumor subtypes. *Cancer informatics*.
- [Gandolfi and Tramontano, 2017] Gandolfi, F. and Tramontano, A. (2017). A computational approach for the functional classification of the epigenome. *Epigenetics and Chromatin*, 10(1):1–24.
- [Goldberg et al., 2007] Goldberg, A. D., Allis, C. D., and Bernstein, E. (2007). Epigenetics: A Landscape Takes Shape.
- [Goll and Bestor, 2005] Goll, M. G. and Bestor, T. H. (2005). Eukaryotic cytosine methyltransferases. *Annual review of biochemistry*.
- [Grewal and Moazed, 2003] Grewal, S. I. and Moazed, D. (2003). Heterochromatin and epigenetic control of gene expression.
- [Hamilton and Baulcombe, 1999] Hamilton, A. J. and Baulcombe, D. C. (1999). A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science*.

- [Heintzman and Ren, 2009] Heintzman, N. D. and Ren, B. (2009). Finding distal regulatory elements in the human genome.
- [Hoffman et al., 2012] Hoffman, M. M., Buske, O. J., Wang, J., Weng, Z., Bilmes, J. A., and Noble, W. S. (2012). Unsupervised pattern discovery in human chromatin structure through genomic segmentation. *Nature methods*.
- [Hon et al., 2008] Hon, G., Ren, B., and Wang, W. (2008). ChromaSig: a probabilistic approach to finding common chromatin signatures in the human genome. *PLoS computational biology*.
- [Hotchkiss, 1948] Hotchkiss, R. D. (1948). The quantitative separation of purines, pyrimidines, and nucleosides by paper chromatography. *The Journal of biological chemistry*.
- [Jeppesen and Turner, 1993] Jeppesen, P. and Turner, B. M. (1993). The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. *Cell*.
- [Jessberger et al., 2007] Jessberger, S., Nakashima, K., Clemenson, G. D., Mejia, E., Mathews, E., Ure, K., Ogawa, S., Sinton, C. M., Gage, F. H., and Hsieh, J. (2007). Epigenetic Modulation of Seizure-Induced Neurogenesis and Cognitive Decline. *Journal of Neuroscience*, 27(22):5967–5975.
- [Johnson et al., 2003] Johnson, J. M., Castle, J., Garrett-Engele, P., Kan, Z., Loerch, P. M., Armour, C. D., Santos, R., Schadt, E. E., Stoughton, R., and Shoemaker, D. D. (2003). Genome-Wide Survey of Human Alternative Pre-mRNA Splicing with Exon Junction Microarrays. *Science*.
- [Jones, 2012] Jones, P. A. (2012). Functions of DNA methylation: Islands, start sites, gene bodies and beyond.
- [Jones and Taylor, 1980] Jones, P. A. and Taylor, S. M. (1980). Cellular differentiation, cytidine analogs and DNA methylation. *Cell*.
- [Kayne et al., 1988] Kayne, P. S., Kim, U. J., Han, M., Mullen, J. R., Yoshizaki, F., and Grunstein, M. (1988). Extremely conserved histone H4 N terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. *Cell*.
- [Kiefer, 2007] Kiefer, J. C. (2007). Epigenetics in development.
- [Kim and Tidor, 2003] Kim, P. M. and Tidor, B. (2003). Subsystem identification through dimensionality reduction of large-scale gene expression data. *Genome Research*.
- [Lee and Seung, 1999] Lee, D. D. and Seung, H. S. (1999). Learning the parts of objects by non-negative matrix factorization. *Nature*.
- [Liu et al., 2004] Liu, L., Lai, S., Andrews, L. G., and Tollefsbol, T. O. (2004). Genetic and epigenetic modulation of telomerase activity in development and disease.
- [Mammana and Chung, 2015] Mammana, A. and Chung, H. R. (2015). Chromatin segmentation based on a probabilistic model for read counts explains a large portion of the epigenome. *Genome Biology*.

- [Mewes et al., 2002] Mewes, H. W., Frishman, D., Güldener, U., Mannhaupt, G., Mayer, K., Mokrejs, M., Morgenstern, B., Münsterkötter, M., Rudd, S., and Weil, B. (2002). MIPS: a database for genomes and protein sequences. *Nucleic acids research*.
- [Mochizuki et al., 2002] Mochizuki, K., Fine, N. A., Fujisawa, T., and Gorovsky, M. A. (2002). Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in Tetrahymena. *Cell*.
- [Partridge et al., 2002] Partridge, J. F., Scott, K. S., Bannister, A. J., Kouzarides, T., and Allshire, R. C. (2002). cis-acting DNA from fission yeast centromeres mediates histone H3 methylation and recruitment of silencing factors and cohesin to an ectopic site. *Current Biology*.
- [Ramakrishna et al., 2012] Ramakrishna, M., Hinton, J., Alexandrov, L., and et al. (2012). Mutational Processes Molding the Genomes of 21 Breast Cancers. *Cell*.
- [Rén et al., 2017] Rén, B., Pueyo, L., Zhu, G. B., Debes, J., and Duchêne, G. (2017). Non-negative Matrix Factorization: Robust Extraction of Extended Structures. *The Astrophysical Journal*, 852(2):104.
- [Schatz et al., 1964] Schatz, G., Haslbrunner, E., and Tuppy, H. (1964). Deoxyribonucleic acid associated with yeast mitochondria. *Biochemical and Biophysical Research Communications*.
- [Sutherland and Costa, 2003] Sutherland, J. E. and Costa, M. (2003). Epigenetics and the environment. In *Annals of the New York Academy of Sciences*.
- [Waddington, 1942] Waddington, C. H. (1942). The epigenotype. *Endeavour*, 1(1):18–20.
- [Wang and Cooper, 2007] Wang, G. S. and Cooper, T. A. (2007). Splicing in disease: Disruption of the splicing code and the decoding machinery.
- [Weber et al., 2005] Weber, M., Davies, J. J., Wittig, D., Oakeley, E. J., Haase, M., Lam, W. L., and Schübeler, D. (2005). Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nature Genetics*, 37(8):853–862.
- [Wray et al., 2007] Wray, N. R., Goddard, M. E., and Visscher, P. M. (2007). Prediction of individual genetic risk to disease from genome-wide association studies. *Genome Research*, 17(10):1520–1528.
- [Xie et al., 2013] Xie, W., Schultz, M. D., Lister, R., and et al. (2013). Epigenomic analysis of multilineage differentiation of human embryonic stem cells. *Cell*.
- [Yang et al., 2007] Yang, Z., Yuan, Z., and Laaksonen, J. (2007). Projective Non-Negative Matrix Factorization with Applications To Facial Image Processing. *International Journal of Pattern Recognition and Artificial Intelligence*, 21(8):1353–1362.