Instructor: Sael Lee
CS549 Spring – Computational Biology

LECTURE 10:
GAUSSIAN MIXTURE MODEL APPLICATION EXAMPLE:
NUCLEOSOME POSITIONING

Reference:
NORMAL: accurate nucleosome positioning using a modified Gaussian mixture model

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ABSTRACT

“Motivation: Nucleosomes are the basic elements of chromatin structure. They control the packaging of DNA and play a critical role in gene regulation by allowing physical access to transcription factors. The advent of second-generation sequencing has enabled landmark genome-wide studies of nucleosome positions for several model organisms. Current methods to determine nucleosome positioning first compute an occupancy coverage profile by mapping nucleosome-enriched sequenced reads to a reference genome; then, nucleosomes are placed according to the peaks of the coverage profile. These methods are quite accurate on placing isolated nucleosomes, but they do not properly handle more complex configurations. Also, they can only provide the positions of nucleosomes and their occupancy level, whereas it is very beneficial to supply molecular biologists additional information about nucleosomes like the probability of placement, the size of DNA fragments enriched for nucleosomes and/or whether nucleosomes are well positioned or ‘fuzzy’ in the sequenced cell sample.

Results: We address these issues by providing a novel method based on a parametric probabilistic model. An expectation maximization algorithm is used to infer the parameters of the mixture of distributions. We compare the performance of our method on two real datasets against Template Filtering, which is considered the current state-of-the-art. On synthetic data, we show that our method can resolve more accurately complex configurations of nucleosomes, and it is more robust to user-defined parameters. On real data, we show that our method detects a significantly higher number of nucleosomes.”
Knowing the precise locations of nucleosomes in a genome is key to understanding how genes are regulated.

Nucleosome positions can tell us about

- How nucleosome positioning distinguish promoter regions and transcriptional start sites, and
- How the composition and structure of promoter nucleosomes facilitate or inhibit transcription.
- How diverse factors, including underlying DNA sequences and chromatin remodeling complexes, influence nucleosome positioning.
Peak calling

Problems of peak calling method
- The coverage profile function has to be cleaned of high-frequency noise,
  - typically via a kernel density estimation method
- peak finding algorithms have parameters (like the extension and the shift discussed above) that are difficult to optimize:
  - a set of parameter can work for a region of a chromosome but not for another
- peak calling do not properly resolve overlapping nucleosomes.
Problem Definition

Problem:

Determine nucleosome positioning and provide information about

- probability of position
- size of DNA fragments enriched for nucleosomes and/or
- whether nucleosomes are well positioned or ‘fuzzy’

NOrMAL: NucleOsome Mapping ALgorithm.
- Parametric probabilistic model for nucleosome positioning
- Uses Expectation Maximization (EM) to infer its parameters.

Conditions to consider
- Although DNA fragments, due to size of nucleosome, be around 146 bp, in reality they are shorter. The digestion process can either leave nucleosome-free DNA in the sample, or ‘over-digest’ the ends of nucleosome-bound DNA.
- The rate of digestion is sequence dependent so nucleosomes in different genomic locations will end up with different DNA fragment size.
Validation

Compare the performance of NOrMAL against the state of the art method in 2011: Template Filtering (TF) algorithm

Dataset:
1. MAINE-seq data for Plasmodium falciparum (Ponts et al., 2010) and
2. MAINE-seq data for Saccharomyces cerevisiae (Weiner et al., 2010)
MAINE-seq data set: micrococcal nuclease digestion of mononucleosomes

Isolates histone-associated DNA
Modified Gaussian mixture model framework: DATA

- $C_i \in [1, K]$: hidden variable representing the nucleosome to which it belongs.

- $i$: any DNA fragment $i \in [1, N]$
- $N$: number of DNA fragments obtained after MNase digestion
- $x_i$: the starting position of the 5'-end of fragment $i$ (obtained by mapping sequenced read)
- $d_i \in \{+1, -1\}$: the strand on which fragment $i$ was mapped (+1 for the positive strand, and -1 for the negative strand).
- $z_i$: the length of fragment $i$. (can be observed directly only if sequencing produces paired-end reads)
- $m_i$: the position of the center of the fragment $i$, $m_i = x_i + \frac{d_i z_i}{2}$

(Fig 2 of A. Polishk et al.) The proposed graphical mixture model: shaded nodes correspond to observed variables, white nodes correspond to hidden variables.

- $X_i, D_i, Z_i$ and $M_i$ the random variables associated with variables $x_i, d_i, z_i$ and $m_i$. 
Each nucleosome $j \in [1, K]$ is described by set of **six variables**

$$\theta = \{\theta_1, ..., \theta_K\}$$

$$\theta_j = (\mu_j, \sigma_j, \Delta_j, \delta_j^{+1}, \delta_j^{-1}, \pi_j)$$

- $\mu_j$ : center position of the nucleosome $j$,
- $\sigma_j$ : the fuzziness associated with the position of nucleosome $j$,
- $\Delta_j$ : describes the length of DNA fragments associated with nucleosome $j$, $(z_i)$
- $\delta_j^{+1}, \delta_j^{-1}$ : variation on fragment site for positive and negative strands, respectively
- $\pi_j$ : is the probability of nucleosome $j$. 

Modified Gaussian mixture model framework: CLASS
* model assumes that our random variables are distributed according to a normal distribution

- assume that variable $M_i$ associated with the center of the fragment $i$ for a particular nucleosome $j$ is distributed as follows

$$P(M_i|C_i = j, \Theta) \sim N(\mu_j, \sigma_j^2)$$

- assume that the length $Z_i$ of fragment $i$ for a particular nucleosome $j$ is distributed as follows

$$P(Z_i|D_i = d_i, C_i = j, \Theta) \sim N(\Delta_j, (\delta_j^{d_i})^2)$$

Assuming the above Gaussians are independent and applying

$$x_i = m_i - \frac{d_i z_i}{2}$$

Probability of a given data point $x_i$ given the parameters of a nucleosome

$$P(X_i|D_i = d_i, C_i = j, \Theta) \sim N\left(\mu_j - \frac{d_i \Delta_j}{2}, \sigma_j^2 + \left(\frac{\delta_j^{d_i}}{2}\right)^2\right)$$

excluded variables $z_i$ from the equation.
Generative Mixture Model

\[ P(X_i|D_i = d_i, C_i = j, \theta) \sim N\left(\mu_j - \frac{d_i \Delta_j}{2}, \sigma_j^2 + \frac{\delta_j d_i}{2}\right) \]

- Likelihood of point \((x_i, d_i)\) given \(\theta\)

\[
P(X_i|D_i = d_i, \theta) = \sum_{j=1}^{K} P(C_i = j|\theta)P(X_i|D_j = d_i, C_i = j, \theta)
\]

\[
= \sum_{j=1}^{K} \pi_j f\left(x_i, \mu_j - \frac{d_i \Delta_j}{2}, \sigma_j^2 + \left(\frac{\delta_j d_i}{2}\right)^2\right)
\]

Where \(f(x, a, b) = \frac{1}{\sqrt{2\pi b}}\exp\left(-\frac{(x-a)^2}{2b}\right)\)
• log likelihood of observed data points $X$ given parameters $\theta$

$$l(X|\theta) = \sum_{i=1}^{N} P(X_i|D_i = d_i, \theta)$$

$$= \sum_{i=1}^{N} \ln \sum_{j=1}^{K} P(C_i = j|\theta) P(X_i|D_j = d_i, C_i = j, \theta)$$

$$= \sum_{i=1}^{N} \ln \sum_{j=1}^{K} \pi_j f \left( x_i, \mu_j - \frac{d_i \Delta_j}{2}, \sigma_j^2 + \left( \frac{d_i \Delta_j}{2} \right)^2 \right)$$

marginal log-likelihood of observed data

$$\ln p(X|\theta) = \ln \{ \sum_{Z} p(X, Z | \theta) \}$$

Expectation of the complete-data log likelihood

$$Q(\theta, \theta^{old}) = \sum_{Z} p(Z | X, \theta^{old}) \ln p(X, Z | \theta)$$

Complete data log likelihood
• estimate the parameters of the model $\theta$ via maximum likelihood
$$\hat{\theta} = \arg\max_{\theta} l(X|\theta)$$

Compute posterior probabilities $P(C_i = j|X_i = x_i; \theta^t)$ of data points $x_i, i \in [1, N]$ w.r.t the distribution of $C_i$ given the current estimate of parameters $\theta^t$

$$Q(\theta|\theta^t) = E_{C|X,\theta^t} l(X|\theta)$$
$$= \sum_c p(C|X, \theta^t) \ln p(X, C|\theta)$$

Find new parameter estimation
$$\theta^{t+1} = \arg\max_{\theta} Q(\theta|\theta^t)$$

the posterior distribution of the latent variables

marginal log-likelihood of observed data
$$\ln p(X|\theta) = \ln\{ \sum_Z p(X, Z|\theta) \}$$

Expectation of the complete-data log likelihood
$$Q(\theta, \theta^{old}) = \sum_Z p(Z|X, \theta^{old}) \ln p(X, Z|\theta)$$
NOrMAL Algorithm

Initialize with non-informative prior
1: \{Parameter initialization\}
2: \( \mu_0 \leftarrow (\mu_1, \mu_2, \ldots, \mu_K) \), where \( \mu_i \) is uniformly distributed
3: \( \pi_0 \leftarrow (\frac{1}{K}, \frac{1}{K}, \ldots, \frac{1}{K}) \) \( \in \mathbb{R}^K \)
4: \( \Theta^{(t)} \leftarrow (\mu_0, \sigma_0, \Delta_0, \delta_0^{+1}, \delta_0^{-1}, \pi_0) \)

5: \{Soft Learning\}
6: \( \Theta \leftarrow \text{Learn}(\Theta^{(t)}) \)

7: \{Hard learning\}
8: \textbf{for all} \( i \in [1, N] \) \textbf{do}
9: \( C_i \leftarrow \text{argmax}_j(T_{ij}) \)
10: \textbf{end for}
11: Recompute cluster parameters \( \Theta \)
12: \textbf{return} \( \Theta \)

One iteration of ‘hard learning’ assigning each data point \( x_i \) its maximum probable cluster

1. Heuristics to find \textbf{number} of nucleosome positions (clusters)
2. Rough estimate of the nucleosome positions.

Learn(\( \Theta \))

1: \textbf{repeat}
2: \textbf{while} not converged \textbf{do}
3: \( \mathbb{Q}(\Theta|\Theta^{(t)}) \leftarrow E_{C|X, \Theta^{(t)}} l(X|\Theta) \)
4: \( \Theta^{(t+1)} \leftarrow \text{argmax}_\Theta \mathbb{Q}(\Theta|\Theta^{(t)}) \)
5: \( t \leftarrow t + 1 \)
6: \textbf{end while}
7: \textbf{for all} \( j \in [1, K - 1] \) \textbf{do}
8: \textbf{if} \( |\mu_j - \mu_{j+1}| \leq \text{threshold} \) \textbf{then}
9: \{Merge clusters \( i \) and \( i - 1 \}\)
10: \textbf{end if}
11: \textbf{end for}
12: \textbf{until} no clusters were merged
13: \textbf{return} \( \Theta^{(t)} \)
Determining the number of Gaussian components

**Heuristics to find K:**

1. **Initialize:** Placing the maximum possible number of non-overlapping nucleosomes uniformly distributed on the chromosome,
   
   \[ K = \frac{\text{size of the chromosome} (\approx 44167 \text{bp})}{\text{expected size of a nucleosome} (\approx 146 \text{bp})} \]
   
   where the expected size of nucleosomes is underestimated.

2. ‘soft learning’: we run our EM algorithm until convergence

3. **merge those that have too much overlap** (distance under a user-specified threshold).
   
   In case of multiple overlaps for a nucleosome, we merge it with the closest one.

4. Repeat (2) and (3) until no additional clusters are merged.

   - After a few cycles, we will obtain a set of non-overlapping clusters that best explain the given data points.
   - Overlapping nucleosomes are merged into new ones and then the position of new nucleosomes are learned from the data.
Template filtering overview:

(A) Deep sequencing data for a typical stretch of the yeast genome.

(B) Templates. Forward and reverse-strand read distributions are cross-correlated with each of the seven templates shown.

(C) Correlation coefficient heat map of template 1 for forward and reverse templates at varying center positions (x-axis) and distances (y-axis).

(D) Examples of templates spaced too far apart (top), at the optimal distance (middle), or too close together (bottom).

(E) Read distributions explained by the optimal template matches are shown as dotted lines for the region in A.

(F) Schematic of nucleosome calls and underlying gene annotations.

Results: Synthetic data

Coverage profile from synthetic data

nucleosomes detected using TF allowing maximum 35% overlap [100,200]

NOrMAL allowing maximum 35% overlap (C)

TF allowing only 30% overlap, [100,200] (D)

NOrMAL allowing only 30% overlap (E);

nucleosome reported by TF with nucleosome size range [40,200] (F)

nucleosome reported by TF with nucleosome size range [100,300] (G)
Results: Real data

Like many bioinformatics problems, the challenge for nucleosome position inference is that the true positions of the nucleosomes are unknown.

-> The author’s have to do their best to convince that reader that their method is better.

<table>
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<th>Chromosome</th>
<th>No of mapped reads</th>
<th>TF</th>
<th>Time (s)</th>
<th>NOrMAL</th>
<th>Time (s)</th>
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1. Validation by agreement between exiting state of the art method
2. Validation by Empirical example