Advanced NGS Analysis (Day 1) Session II

Lyda Hill department of Bioinformatics 2022 Nanocourse Series

Date & Time: June 27-28: 9AM-5PM (NG3.202)

Course Instructors: Bo Li, Daehwan Kim, Christopher Chaney, & **Micah Thornton**

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Day 2: RNA-Seq Analysis Using Pseudo/Quasi-Alignment and Expectation Maximization (Kallisto, Salmon, H2Q).

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What you will learn in this Session (2 Parts)

- *Day 1*
- *Some Theoretical Considerations:*
    - *What is Pseudo/Quasi-Alignment*
        - *What is Alignment*
- *Some Practical Considerations:*
    - *What is Kallisto*
    - *What is Salmon*
    - *What is H2Q*
- *Day 2*
    - *What is Expectation Maximization for Gene Transcript Quantification*
    - *What is the resolution of Genetic Transcript Data?*
    - *What is Expectation Maximization*

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Part 1: Pseudo and Quasi Alignment & Quantification Resolution

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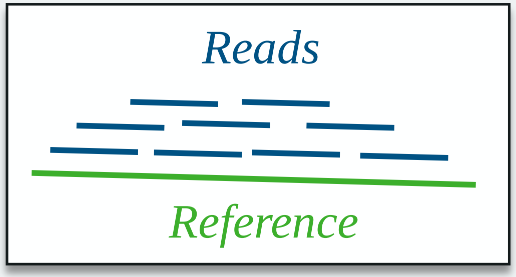
RNA-Seq Experiments

- RNA-Seq experiments are fundamentally distinct from DNA-seq experiments, and seeks to answer a different set of questions.
- Usually we are seeking to determine whether the level of expression of a particular gene is related to a phenotypic characteristic of interest.

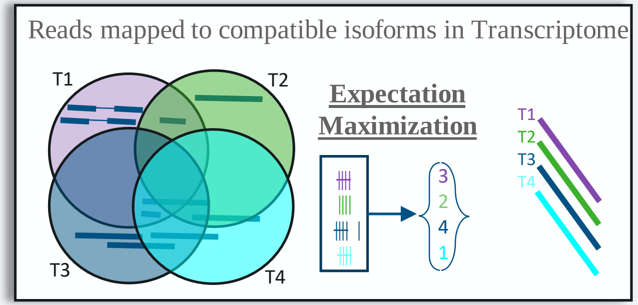
Pseudo/Quasi Alignment in RNA Experiments

- Sometimes the *exact* position of a sequencing read is not of critical import.
  - There are a few approaches for resolving the *approximate* location of a read.
  - Procedures work by determining the subset of *transcript isoforms* compatible with a read.
  - Two such approaches are known as:
    - Pseudo-Alignment
      - The Approach used by **Kallisto.**
      - Uses the De Brujin (‘Deh-Broine’) graph procedure.
    - Quasi-Alignment
      - The Approach used by **Salmon.**
      - Uses a *K*-mer Hash table and Suffix Array.

Typical ‘DNA-Seq Like’ Experiment



Typical ‘RNA-Seq Like’ Experiment



Recall that in most typical sequencing experiments we are dealing with a large collection of shorter subsequences called ***reads***, which we attempt to map to a larger sequence known as the ***reference***.

Resources – Kallisto (Pseudo-alignment)

1. https://tinyheero.github.io/2015/09/02/pseudoalignments-kallisto.html (Higher Level Overview pseudo alignment)
2. https://www.youtube.com/watch?v=f-ecmECK7lw (Video Describing how To Build The De Brujin graph)
3. https://www.nature.com/articles/nbt.2023 (Nature Primer on Using De Brujin Graphs for Genomic Alignments).

Resources – Salmon (Quasi-alignment)

1. https://hbctraining.github.io/Intro-to-rnaseq-hpc-salmon-flipped/lessons/08_quasi_alignment_salmon.html (Higher Level Overview Quasi-Alignment)
2. https://academic.oup.com/bioinformatics/article/32/12/i192/2288985?login=true (RapMap Paper and Description).

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Part 2: Expectation Maximization & Gene Transcript Quantification

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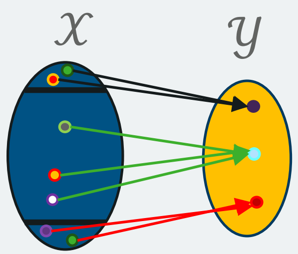
Expectation Maximization (in general) – Incomplete Data & A Restricted Case

Many-to-one relationship



- Two general uses include:
  - determination of maximum likelihood estimates for parameters when missing data is present and
  - estimation of missing or otherwise incomplete data.

- In general, suppose that we would like to observe the values, $x_1, x_2, \dots x_n$, to determine something about the parameters of the random variable X which has sample space $\mathcal{X}$ as shown (top right).
  - However, we are only able to observe, $y_1, y_2, \dots y_n$, valuations of the random variable Y which has sample space $\mathcal{Y}$ onto which there exists a many-to-one mapping from $\mathcal{X}$.
    - In other words, there are multiple values possible to observe in $\mathcal{X}$ corresponding to the same value in $\mathcal{Y}$.

- Suppose, at first, that the distribution of $\boldsymbol{X}$ (note boldface indicates that $\boldsymbol{X}$ could be a vector quantity) is one of the exponential family of distributions generally denoted,

$$f_{\boldsymbol{X}}(\boldsymbol{x}|\boldsymbol{\theta}) = b(\boldsymbol{x})e^{\left(\boldsymbol{\theta t}(\boldsymbol{x})^{\boldsymbol{T}}\right)}a(\boldsymbol{\theta})^{-1}$$

$\boldsymbol{\theta}$ is a parameter [column]-vector (of size r).
$\boldsymbol{t}(\boldsymbol{x})^{\boldsymbol{T}}$ is the sufficient statistic [row]-vector (of size r).
$a(\cdot), b(\cdot)$, are any arbitrary function.
e is the natural number.

See section II of the Dempster, Laird, Rubin paper mentioned below for more details about natural parameters.

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These Expectation Maximization Notes Draw Heavily from “Maximum Likelihood from Incomplete Data via the EM Algorithm” by Dempster Rubin and Laird (https://www.jstor.org/stable/2984875)

Expectation Maximization (in general) – The Algorithm

- The "simple characterization" of the EM algorithm according to Dempster, Laird, and Rubin (DLR77) is:

(1) With $\theta^{(p)}$ indicating the estimate of θ at the p^{th} step of the algorithm, estimate the complete-data sufficient statistics $\boldsymbol{t}(\boldsymbol{x})$ by finding

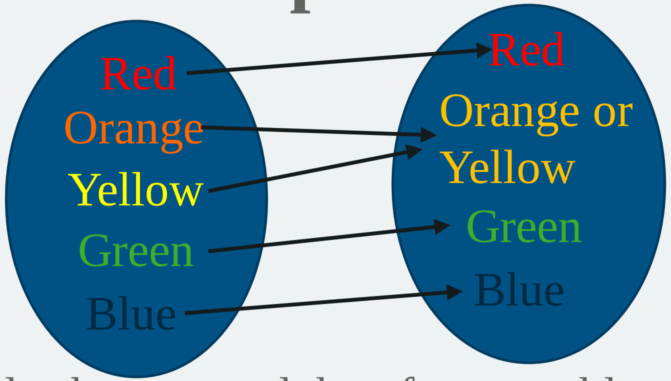
$$\boldsymbol{t}^{(p)} = E\left(\boldsymbol{t}(\boldsymbol{x})\middle|\boldsymbol{y},\boldsymbol{\theta}^{(p)}\right).$$

(2) Perform maximum likelihood estimation to determine $\theta^{(p+1)}$ from $\boldsymbol{t}^{(p)}$,

$$\boldsymbol{E}(\boldsymbol{t}(\boldsymbol{x})|\boldsymbol{\theta}) = \boldsymbol{t}^{(p)}.$$

- Proof of convergence to the maximum likelihood value is given the DLR77, as are details regarding further generalizations of the expectation maximization algorithm.
- The algorithm is broadly applicable in many cases, and not all of the applications have been discovered yet.

Expectation Maximization (in general) – A Multinomial Example



- Suppose that there are marbles of five colors in a bag.
  - Red marbles are denoted by ‘R’
  - Orange marbles are denoted by ‘O’
  - Yellow marbles by ‘Y’
  - Green marbles by ‘G’
  - Blue marbles by ‘B’
- Now, you personally cannot tell a difference between the orange and the yellow marbles by eye, and therefore are able to produce counts of four categories of marbles only (that is: “Red”, “Orange or Yellow”, “Green”, and “Blue”).

[EXAMPLE]

- Suppose it is known ahead of time that the proportions of the *actual* colors of each of the marbles are related via an unknown parameter π, such that for the unobservable true color of an arbitrarily selected marble i, denoted c_i (true color) given below induces a distribution on the observable o_i (observed color) follows this distribution:

$$P\left(c_i = \begin{pmatrix} \text{Red} \\ \text{Orange} \\ \text{Yellow} \\ \text{Green} \\ \text{Blue} \end{pmatrix}\right) = \begin{pmatrix} (1-\pi)/4 \\ \pi/4 \\ 1/2 \\ (1-\pi)/4 \\ \pi/4 \end{pmatrix} \Rightarrow P\left(o_i = \begin{pmatrix} \text{Red} \\ \text{Orange or Yellow} \\ \text{Green} \\ \text{Blue} \end{pmatrix}\right) = \begin{pmatrix} (1-\pi)/4 \\ 1/2 + \pi/4 \\ (1-\pi)/4 \\ \pi/4 \end{pmatrix}$$

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Expectation Maximization (in general) – A Multinomial Example (Continued)

Suppose that we observe 197 marbles, and arrive at the following counts:

$$\begin{pmatrix} \text{R}-\text{Red: 18} \\ \text{OY}-\text{Orange or Yellow:125} \\ \text{G}-\text{Green: 20} \\ \text{B}-\text{Blue: 34} \end{pmatrix}$$

$$P\left(c_i = \begin{pmatrix} \text{Red} \\ \text{Orange} \\ \text{Yellow} \\ \text{Green} \\ \text{Blue} \end{pmatrix}\right) = \begin{pmatrix} (1-\pi)/4 \\ \pi/4 \\ 1/2 \\ (1-\pi)/4 \\ \pi/4 \end{pmatrix} \Rightarrow P\left(c_i = \begin{pmatrix} \text{Red} \\ \text{Orange or Yellow} \\ \text{Green} \\ \text{Blue} \end{pmatrix}\right) = \begin{pmatrix} (1-\pi)/4 \\ 1/2+\pi/4 \\ (1-\pi)/4 \\ \pi/4 \end{pmatrix}$$

$$x_j = \sum_{i=1}^{197} \mathbb{1}(c_i \equiv j) \qquad j \in \begin{pmatrix} \text{Red} \\ \text{Orange} \\ \text{Yellow} \\ \text{Green} \\ \text{Blue} \end{pmatrix}$$

$$y_t = \sum_{i=1}^{197} \mathbb{1}(o_i \equiv t) \qquad t \in \begin{pmatrix} \text{Red} \\ \text{Orange or Yellow} \\ \text{Green} \\ \text{Blue} \end{pmatrix}$$

- Let the *actual* color counts be denoted by the values $(x_1, x_2, x_3, x_4, x_5)$ such that x_1 corresponds to the count of marbles which were actually red, x_2 to those which were Orange, and so on…
- Let the observed color counts be denoted by the values (y_1, y_2, y_3, y_4) which are given in this example as (18,125,20,34).
- Furthermore, it is known that $y_2 = x_2 + x_3$.

- The Likelihood on π for the full data can be expressed as:

$$f(\boldsymbol{x}|\pi) = \frac{\left(\sum_{i=1}^{5} x_i\right)!}{\prod_{i=1}^{5}(x_i!)} \cdot \left(\frac{1-\pi}{4}\right)^{x_1} \cdot \left(\frac{\pi}{4}\right)^{x_2} \cdot \left(\frac{1}{2}\right)^{x_3} \cdot \left(\frac{1-\pi}{4}\right)^{x_4} \cdot \left(\frac{\pi}{4}\right)^{x_5}$$

- The *coarsened/incomplete* Likelihood on π for the full data can be expressed as:

$$g(\boldsymbol{y}|\pi) = \frac{\left(\sum_{i=1}^{4} y_i\right)!}{\prod_{i=1}^{4}(y_i!)} \cdot \left(1-\frac{\pi}{4}\right)^{y_1} \cdot \left(\frac{1}{2}+\frac{\pi}{4}\right)^{y_2} \cdot \left(1-\frac{\pi}{4}\right)^{y_3} \cdot \left(\frac{\pi}{4}\right)^{y_4}$$

DLR77 (https://www.jstor.org/stable/2984875)

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Expectation Maximization (in general) – A Multinomial Example (E-Step)

- Clearly, due to the fact that a marble cannot *actually* be two colors simultaneously, there is no probability that any marble is *truly* both orange and yellow at the same time, therefore we may express the probability that a marble is orange or yellow as follows:

$$P\left(o_i = (\text{Orange or Yellow})\right) = P\left(c_i \in \binom{\text{Orange}}{\text{Yellow}}\right) = P(c_i = \text{Orange}) + P(c_i = \text{Yellow}) - P(c_i = \text{Yellow} \;\&\; c_i = \text{Orange})$$

$$= P(c_i = \text{Orange}) + P(c_i = \text{Yellow}) - 0 = \frac{\pi}{4} + \frac{1}{2}$$

- From here we can derive the expression for the maximum likelihood estimates of the unobserved counts for orange and yellow marbles (x_2, x_3) in terms of the observed count of "orange or yellow" marbles (y_2).

$$P\left(c_i = \text{Orange} \,|\, o_i = (\text{Orange or Yellow})\right) = \frac{P(o_i = (\text{Orange or Yellow}) \;\&\; c_i = \text{Orange})}{P(o_i = (\text{Orange or Yellow}))} = \frac{P(c_i = \text{Orange})}{P\left(o_i = (\text{Orange or Yellow})\right)} = \frac{\frac{\pi}{4}}{\frac{\pi}{4} + \frac{1}{2}}$$

$$P\left(c_i = \text{Yellow} \,|\, o_i = (\text{Orange or Yellow})\right) = \frac{\frac{1}{2}}{\frac{\pi}{4} + \frac{1}{2}}$$

Therefore the conditional expectation of x_2 and x_3 are:

$$E(x_2|y_2) = y_2 \frac{\frac{\pi}{4}}{\frac{\pi}{4} + \frac{1}{2}} \quad \text{- and -} \quad E(x_3|y_2) = y_2 \frac{\frac{1}{2}}{\frac{\pi}{4} + \frac{1}{2}}$$

Suppose that we observe 197 marbles, and arrive at the following counts:

$$\begin{pmatrix} \text{R} - \text{Red: } 18 \\ \text{OY} - \text{Orange or Yellow:} 125 \\ \text{G} - \text{Green: } 20 \\ \text{B} - \text{Blue: } 34 \end{pmatrix}$$

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Expectation Maximization (in general) – A Multinomial Example (M-Step)

$$\Rightarrow x_1 + x_4 = x_2\hat{\pi} + x_5\hat{\pi} + x_1\hat{\pi} + x_4\hat{\pi}$$

- Recall that the full likelihood for the multinomial distribution was given by:

$$f(\boldsymbol{x}|\pi) = \frac{\left(\sum_{i=1}^{5} x_i\right)!}{\prod_{i=1}^{5}(x_i!)} \cdot \left(\frac{1-\pi}{4}\right)^{x_1} \cdot \left(\frac{\pi}{4}\right)^{x_2} \cdot \left(\frac{1}{2}\right)^{x_3} \cdot \left(\frac{1-\pi}{4}\right)^{x_4} \cdot \left(\frac{\pi}{4}\right)^{x_5}$$

$$\Rightarrow \log L(\pi|\boldsymbol{x}) = \log\frac{\left(\sum_{i=1}^{5} x_i\right)!}{\prod_{i=1}^{5}(x_i!)} + x_1 \log\left(\frac{(1-\pi)}{4}\right) + x_2 \log\left(\frac{\pi}{4}\right) + x_3 \log\left(\frac{1}{2}\right) + x_4 \log\left(\frac{(1-\pi)}{4}\right) + x_5 \log\left(\frac{\pi}{4}\right)$$

- In this example, only x_2 and x_3 are unobservable, the rest are known:

$$\Rightarrow \frac{\partial \log L(\pi|\boldsymbol{x})}{\partial\, \pi} = x_1\left(\frac{4}{1-\pi}\right)\left(-\frac{1}{4}\right) + x_2\left(\frac{4}{\pi}\right)\left(\frac{1}{4}\right) + x_4\left(\frac{4}{1-\pi}\right)\left(-\frac{1}{4}\right) + x_5\left(\frac{4}{\pi}\right)\left(\frac{1}{4}\right) = \frac{x_1}{\pi-1} + \frac{x_2}{\pi} + \frac{x_4}{\pi-1} + \frac{x_5}{\pi}$$

$$\Rightarrow \frac{x_1}{\hat{\pi}-1} + \frac{x_2}{\hat{\pi}} + \frac{x_4}{\hat{\pi}-1} + \frac{x_5}{\hat{\pi}} = 0 \Rightarrow (x_2 + x_5)(1-\hat{\pi}) = (x_1 + x_4)\hat{\pi} \Rightarrow x_2 + x_5 - x_2\hat{\pi} - x_5\hat{\pi} = x_1\hat{\pi} + x_4\hat{\pi}$$

$$\Rightarrow x_2 + x_5 = (x_1 + x_2 + x_4 + x_5)\hat{\pi} \Rightarrow \hat{\pi} = \frac{(x_2 + x_5)}{x_1 + x_2 + x_4 + x_5}$$

$$\Rightarrow -x_1\hat{\pi} - x_4\hat{\pi} + x_1 + x_4 = x_2\hat{\pi} + x_5\hat{\pi}$$

$$\Rightarrow x_1 + x_4 = x_2\hat{\pi} + x_5\hat{\pi} + x_1\hat{\pi} + x_4\hat{\pi}$$

Suppose that we observe 197 marbles, and arrive at the following counts:

$$\begin{pmatrix} x_1 \\ x_4 \\ x_5 \end{pmatrix} = \begin{pmatrix} 18 \\ 20 \\ 34 \end{pmatrix} \Rightarrow \hat{\pi} = \frac{x_2 + 34}{18 + x_2 + 20 + 34}$$

$$\begin{pmatrix} \text{R}-\text{Red: } 18 \\ \text{OY}-\text{Orange or Yellow:}125 \\ \text{G}-\text{Green: } 20 \\ \text{B}-\text{Blue: } 34 \end{pmatrix}$$

$$\therefore \frac{1}{\hat{\pi}} = \frac{18 + x_2 + 20 + 34}{x_2 + 34} = 1 + \frac{38}{x_2 + 34} \Rightarrow \hat{\pi} = \frac{1}{1 + \frac{38}{x_2 + 34}}$$

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Expectation Maximization (in general) – A Multinomial Example (Iteration)

- Taking the conditional expectations for the computation of x_2 and x_3 will depend on a particular estimation of π, an initial estimate ($\pi^{(0)}$) must be supplied to the algorithm to start the procedure, then conditional expectations for the missing (coarsened) data at the p^{th} step (where $p \in \{1,2,\ldots\}$) is given by:

[E – Step]

$$E_{(p)}(x_2|y_2) = y_2 \frac{\frac{\pi^{(p-1)}}{4}}{\frac{\pi^{(p-1)}}{4} + \frac{1}{2}} \quad \text{- and -} \quad E_{(p)}(x_3|y_2) = y_2 \frac{\frac{1}{2}}{\frac{\pi^{(p-1)}}{4} + \frac{1}{2}}$$

[M – Step]

$$\widehat{\pi^{(p)}} = \frac{1}{1 + \frac{38}{E_{(p)}(x_2|y_2) + 34}}$$

- Convergence Criteria:
  - Generally we use relative convergence criteria (when the change in the parameters from step p to step $p+1$ falls below a relative tolerance ε_R) to determine when to stop iterating, for instance, the iteration will continue until:

[Convergence]

$$\left(\frac{1}{1 + \frac{38}{E_{(p)}(x_2|y_2) + 34}} - \frac{1}{1 + \frac{38}{E_{(p-1)}(x_2|y_2) + 34}} \right)^2 \leq \varepsilon_R$$

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Expectation Maximization (Genetic Abundance Estimation)