

Written Report

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Final assignment written report.

MDGE 610: Foundations of Bioinformatics.

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This document contains the responses for the four question of the assignment including reproducibility code when applicable. The overall github repository containing resulting files, individual codes, and track changes can be found at the following link: [MDGE610_final_project](#).

First Section. Conceptual understanding

The EM algorithm.

What is the EM algorithm?

The expectation maximization algorithm is a general approach for the iteration of computational calculations of maximum likelihood estimates as the function of the observed data. This allows for the optimization of the log-likelihood values for observed data as well as ‘incomplete data’, where the calculation of maximum likelihood estimate is complex due to missing variables or weights unbalance. For instance, linear regression represents a statistical approach to understand the association of several variables into an specific outcome as modeled of a linear function. The effect of each variable is represented by a coefficient that decreases the sum of squares residuals (discrepancy between observed data and model prediction). These measure are commonly optimized by the least-squares estimation. Under expectation maximization algorithm, the influence of missing data can be expected based on the observed data (expectation; E-step) and least-squares estimation can be performed using the re-calculated variables (maximization, M-step). Overall, the expectation maximization algorithm favors estimation of statistics for models with incomplete data.

What objective function does it maximize?

The expectation maximization algorithm aims to maximize the log-likelihood function of the observed data; the statistical model underlying the maximum likelihood estimation. This is a measure of how the statistical model explain the observed data. In simpler words, it is the probability of seeing the observed data given the model. This function is commonly used in the frequentist statistics for reducing errors such as the least-squares estimation from linear regression modelling.

What are the E-step and M-Step, conceptually?

The expectation step or E-step corresponds to the section of the algorithm that allows for the estimation of the sufficient statistics of the complete data based on the observed data. Sufficient statistics represents all the information that can be obtained from a dataset; thus, the E-step aims to calculate all the possible information from the observed data for the complete data filling for missing values. Furthermore, the maximization step or M-step represents the recalculation of the maximum likelihood function (log-likelihood) by using the expected complete data instead of the observed data. This way, it allows for maximizing the objective function with the completed data ignoring the constraints of the missing values.

What constitutes the missing data in kallisto's application of EM?

The missing data of the kallisto software is the equivalent classes of the transcript. An equivalent class is a multi-set of transcripts associated with a read indicating which transcript the read could have originated from. This provides the identity of the read as well as their overlap with the reference (E-step), providing enough information (sufficient statistics) for abundance estimation (M-step).

kallisto's Objective Function

What is the mathematical form of the objective function

The mathematical function that represents the likelihood function for the abundance quantitation of the RNA sequencing is:

$$L(\alpha) \propto \prod_{f \in F} \sum_{t \in T} y_{f,t} \frac{\alpha_t}{l_t} = \prod_{e \in E} \left(\sum_{t \in e} \frac{\alpha_t}{l_t} \right)^{c_e}$$

Figure 1: likelihood function for RNA-seq (Bray et al. 2016)

Here, F and T represent the total set of fragments (f) and transcripts (t), respectively. l_t is the effective length of transcript t (length with pseudoalignment) and $y_{f,t}$ is the compatibility matrix (where 1 is compatibility/pseudoalignment between f and t and 0 equals no compatibility). Finally, α_t represents the probability of selecting fragments from transcripts. Overall, this formula represents the product over equivalence classes, with c_e being the number of counts observed of equivalence class e .

How does kallisto's EM implementation maximize it

The expectation maximization algorithm implemented by kallisto aims to expect the equivalence classes (the transcript from which the read might come from) by maximizing the probability of selecting a fragment from a specific transcript α_t . This means that it optimizes the chances of obtaining a read to be associated to an specific transcript, which would serve as the needed information for estimation of their respective abundance.

Another advantage of kallisto is that the EM-optimized likelihood function is defined in term of equivalent classes. This allows for a significant reduction in the dataset that needs to be analyze, resulting in a faster software in comparison to previous tools that conduct alignments.

What convergence criteria does kallisto use to decide when to stop iterating? Where are these specified in the code

The convergence criteria for stopping the iterations is determine by the total expected value of fragment belonging to an specific transcript ($\alpha_t N$). Authors define the end of the iterations as for when every transcript t , the total expected value is above 0.01 allowing for a change less than 1% iteration to iteration. In simpler words, the iterations of the expectation maximization algorithm stop when all of the transcripts have an minimum 0.01 abundance and its variance is less than 1% between iterations.

This specification is stated in the source code for the EM algorithm of the kallisto software. The file can be found at the directory `/kallisto/src/EMAlgorithm.h`. The specific location of this convergence criteria can be found in the lines

Here, this coding represents []

How might these criteria be justified

IT IS FUCKING ALBITRARY IF U ASK ME

Local vs. Global Maxima

Examine how kallisto initializes its abundance estimates

[]

Do you think initialization matter for this problem? Why or why not?

□

Second Section. Empirical investigation

Testing Convergence Criteria

When you click the **Render** button a document will be generated that includes both content and the output of embedded code. You can embed code like this:

```
1 + 1
```

```
[1] 2
```

You can add options to executable code like this

```
[1] 4
```

The `echo: false` option disables the printing of code (only output is displayed).