Simcluster: clustering enumeration gene expression data on the simplex space

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Abstract

Transcript enumeration methods such as SAGE, MPSS, and sequencing-by-synthesis EST “digital northern”, are important high-throughput techniques for digital gene expression measurement. As other counting or voting processes, these measurements constitute compositional data exhibiting properties particular to the simplex space where the summation of the components is constrained. These properties are not present on regular Euclidean spaces, on which hybridization-based microarray data is often modeled. Therefore, pattern recognition methods commonly used for microarray data analysis may be non-informative for the data generated by transcript enumeration techniques since they ignore certain fundamental properties of this space. Here we present a software tool, Simcluster, designed to perform clustering analysis for data on the simplex space. We present Simcluster as a stand-alone command-line C package and as a user-friendly on-line tool. Both versions are available at: \url{http://xerad.systemsbiology.net/simcluster/}. Simcluster is designed in accordance with a well-established mathematical framework for compositional data analysis, which provides principled procedures for dealing with the simplex space, and is thus

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applicable in a number of contexts, including enumeration-based gene expression data.

Background

Technologies for high-throughput measurement of transcriptional gene expression are mainly divided into two categories: those based on hybridization, such as all microarray-related technologies [24, 18] and those based on transcript enumeration, which include SAGE [29], MPSS [13], and Digital Northern powered by traditional [22] or, recently developed, EST sequencing-by-synthesis (SBS) technologies [11].

Currently, transcript enumeration methods are relatively expensive and more time-consuming than methods based on hybridization. However, recent improvements in sequencing technology, powered by the “$1000 genome” effort [26], promises to transform the transcript enumeration approach into a fast and accessible alternative [21, 25, 12] paving the way for a systems-level absolute digital description of individualized samples [20].

Methods for finding differentially expressed genes have been developed specifically in the context of enumeration-based techniques of different sequencing scales such as EST [10], SAGE [31] and MPSS [27]. However, in spite of their differences, hybridization-based and enumeration-based data are typically analyzed using the same pattern recognition techniques, which are generally imported from the microarray analysis field.

In the case of clustering analysis of gene profiles, the simple appropriation of practices from the microarray analysis field has been shown to lead to suboptimal performance [14]. Cai and co-workers [14] provided an elegant clustering computational solution to group tag (genes) profiles that takes into account the specificities of enumeration-based datasets. However, to the best of our knowledge, a solution for transcript enumeration libraries is still needed. We report on a novel computational solution, called Simcluster, to support clustering analysis of transcript enumeration libraries.
Implementation

Theory

Without loss of generality, we use the term “tag” to refer to the transcripts’ representation, as usual in the SAGE field (this is equivalent to the term “signature” in MPSS analysis or “contigs” in EST analysis).

The theoretical model used here to describe the transcript enumeration process is the usual uniform sampling of interchangeable colored balls from an infinite urn model. Given the total number \( n \) of counted tags and the abundance vector \( \pi \) of all transcripts, this model leads to a probabilistic description of the observed result: \( x|\pi, n \sim \text{Multi}(\pi, n) \), i.e., the counts \( x \) follow a Multinomial distribution \[30]. It is also possible to model \( x \) as Poisson distributed \[28\] since it is an approximation for the Multinomial.

Regardless of the specificities of the theoretical probabilistic model, it is well known that, as with other counting or voting processes, the natural space for dealing with this kind of data is the simplex space.

The unitary simplex space, having \( d \) dimensions, is defined as \[8, 9\]:

\[
S_{d-1} = \{ \pi | \pi \in \mathbb{R}^d_+, \pi 1' = 1 \}
\]

where \( 1 \) is a vector of ones. In the gene expression context, \( d \) is the number of unique tags observed.

An example of a simplex vector is \( p = \mathbb{E}[\pi|x] \) and applying a standard Bayesian approach, one obtains from \( x|\pi, n \), using a Dirichlet prior density \( \pi \sim \text{Dir}(\alpha) \), the posterior density: \( \pi|x \sim \text{Dir}(x + \alpha) \).

It is known that clustering analysis is inherently dependent on the choice of a distance measure between the considered objects. This, in turn, is connected to the structure of the underlying space. A metric \( \Delta \), measuring the distance between two objects \( a \) and \( b \), must respect the properties:

(i) \( \Delta(a, b) = \Delta(b, a) \);
(ii) \( \Delta(a, b) = 0 \iff a = b \);
(iii) \( \Delta(a, c) \leq \Delta(a, b) + \Delta(b, c) \).

One may also consider additional reasonable properties such as:

(iv) scale invariance \( \Delta(xa, yb) = xy\Delta(a, b), x, y \in \mathbb{R}; \) or
(v) translational invariance \( \Delta(a + t, b + t) = \Delta(a, b) \).

Translations on the simplex space are defined by \[9\]:

\[3\]
\[ p \oplus t = \frac{(p \cdot t)}{(p \cdot t)\mathbf{1}'} \]

(2)

where \( \cdot \) is the usual Hadamard product and the division is vector-evaluated.

Well known distances, such as Euclidean, Manhattan, and correlation-based distances, do not exhibit the metric properties (i)-(v) if the measured objects belong to the simplex space, as is the case of transcript enumeration data. A metric that obeys (i)-(v) on the simplex space is the Aitchisonian distance [9]:

\[
\Delta(p, q) = \sqrt{\ln \left( \frac{p_{-d}/p_d}{q_{-d}/q_d} \right) \left( I + 1' \times 1 \right)^{-1} \ln \left( \frac{p_{-d}/p_d}{q_{-d}/q_d} \right)'}
\]

(3)

where \( I \) is the identity matrix, \( \times \) is the Kronecker product, \(-d\) subscript is a notation for “excluding the \( d^{th}\) element”, and elementary operations are vector-evaluated.

Clustering procedures coherent with this theoretical background are suitable for transcript enumeration data.

Software design

In short, Simcluster’s method can be described as the use of a Bayesian inference step (currently with a uniform prior) to obtain the expected abundance simplex vectors given the observed counts \( \mathbb{E}[\pi|x] \), and the use of the Aitchisonian distance in the following algorithms: k-means, k-medoids and self-organizing maps (SOM) for partition clustering, PCA for inferring the number of variability sources present, and common variants of agglomerative hierarchical clustering.

Currently, the Simcluster package is comprised of: Simtree, for hierarchical clustering; Simpart, for partition clustering; Simpca for Principal Component Analysis (PCA); and several utilities such as TreeDraw, a program to draw hierarchical clustering dendrograms with user-defined colored leaves. Simcluster’s modularity allows relatively simple extension and addition of new modules or algorithms. Increasing the coverage of algorithms is envisioned in future updates.

Simcluster can be used, modified and distributed under the terms of the GPL license [3]. The software was implemented in C for improved performance and memory usage, assuring that even large datasets can be processed on a regular desktop PC.
To increase source code reuse, established libraries were used: Cluster 3 [16] for clustering, GNU Scientific library [4] for PCA, Cairo [1] and a modification of TreeDraw X [23] for colored dendrogram drawing.

The input data set can be a matrix of transcript counts or general simplex vectors. Some auxiliary shell and Perl scripts are available to: automatically download data from the GEO database [2], convert GEO files to Simcluster input format, and filter out low-count tags.

The Linux-based installation and compilation is facilitated by a configuration script that detects all the prerequisites for Simcluster compilation. Missing libraries are automatically downloaded from the Simcluster website and compiled by the Simcluster compilation process.

To broaden usability, a user-friendly web interface was developed and is made available at http://xerad.systemsbiology.net/simcluster/. Figure 1 shows a screenshot of an analysis session using Simcluster’s web-based interface.

Results and Discussion

We agree with Dougherty and Brun [17] that “validation” of clustering results is a heuristic process. However, to illustrate the usefulness of our software, we collected several examples in which the performance of Simcluster can be considered as qualitatively superior to some traditional approaches imported from the microarray analysis field. These examples include EST, SAGE and MPSS datasets, and are available on the project’s webpage [7]. Among these, we describe here one virtual enumeration dataset build based on real microarray data and a SAGE transcript enumeration dataset.

The objective of the first example is to show that Simcluster is able to reconstruct the clustering result obtained for an Affymetrix microarray dataset when the input is a simulated transcript enumeration dataset, built to mimic the real microarray biological data.

The data used to create the virtual transcript enumeration data was obtained from the Innate Immunity Systems Biology project [5] and is provided as an Additional File. This data is a set of Affymetrix experiments of mouse macrophages stimulated by different Toll-like receptor agonists (LPS, PIC, CPG, R848, PAM) during a time-course (0, 20, 40, 60, 80 and 120 minutes). A detailed description and biological significance of this dataset is presented.
Using this data, a clustering analysis result is shown in Figure 2. This pattern is obtained using the most common type of clustering analysis in the microarray field: Euclidean distance with complete linkage agglomerative hierarchical clustering, implemented by R [6] routines, available as Additional File. This clustering pattern will be considered to be the “gold-standard” for the purpose of this simulation.

The virtual experiment consists of the creation of a transcriptome with the relative abundance between genes defined by the Affymetrix data; sampling a random number of tags from it of different magnitudes; enumeration of sampled transcripts; and using some common clustering procedures along with Simcluster.

It is easier to understand the concept of the virtual transcriptome by following a particular case. For the sample labeled LPS-120 measured 120 minutes after the LPS stimulus, the Affymetrix expression levels are:

| Probesets | Representative ID | Gene Symbol          | Intensity (sorted) |
|-----------|-------------------|----------------------|--------------------|
| 1457375_at| BG094499          | Transcribed locus    | 1.94760            |
| 1452109_at| BG973910          | interleukin 17 receptor E | 2.14522           |
| ...      | ...               | ...                  | ...               |
| M124813_at| AFFX-b-ActinMur   | actin beta cytoplasmic | 36191.41765       |
| 1436996_x_at| AV066625    | lysozyme structural  | 43458.17590       |

The virtual total number of available tags is defined as proportional to the measured intensity using 10,000 as a scaling constant, an arbitrary number large enough to assure that finite population issues are negligible. Actual examples are: 19,476 for BG094499; 21,452 for BG973910; and so on until 361,914,176 for actin; and 434,581,759 for AV066625. The total amount of available tags is $T = 126,971,909,452$, which is a number much greater than the typical number of sequenced tags and is in accordance with the “infinite urn” model.

The total of virtually sequenced tags $N$ for each sample is simulated from a Poisson distribution, $N \sim \text{Poisson}(n)$, to create a realistic virtual sequencing library. All generated data and results are available as Additional Files. For example, the actual simulation for $n = 1,000,000$ virtually sequenced tags assigned $N = 1,001,794$ for the LPS-120 library; $N = 998,382$ for the
CPG-40 library; and so on. The same process is repeated for increasing $n$ from 100,000 to 100,000,000. Since $n \ll T$ for all $n$ considered, the multinomial sampling is used and its mean is taken for each library, according to the assumed “infinite urn” model. The results for the largest simulation are shown in Figures 3-6 and individual results for all separate increasing $n$ sizes are available as Additional Files.

It is clear that cluster results obtained by Simcluster converge to the same structure obtained by analyzing the Affimetrix data, as the number of virtually sequenced tags increases. Moreover, Simcluster’s results are not only compatible with the usual microarray analysis for Affymetrix data, but also are more biologically meaningful than the results obtained by the usual microarray analysis techniques applied to the virtual sequencing data. As in the original microarray analysis, the Simcluster result is able to cluster together the different stimuli, placing consecutive time-points close to each other. The exception is for the PIC, R848 and CPG samples, taken at 120 minutes, which interestingly appear clustered together rather than with their other time-point counterparts (even in the original microarray data).

The second example consists of real data analysis using SAGE transcript enumeration data presented by Chabardes-Garonne and co-workers [15] and publicly available in the GEO database [2] under the accession number GSE694.

In this work, the authors studied the expression profile of several sections of normal kidney. They selected 773 differentially expressed tags using the criteria $P$-value < 0.01 and fold-change $\geq$ 7-fold. Figures 7-10 show a cluster analysis output using these genes. The kidney regions used are: Glomerulus (Glom), proximal convoluted tubes (PCT), proximal straight tubule (PST), medullary thick ascending limb of Henle’s loop (MTAL), cortical thick ascending limb of Henle’s loop (CTAL), cortical collecting duct (CCD), outer medullary collecting duct (OMCD), and distal convoluted tubule (DCT).

Simcluster is able to reconstruct the same conclusion of the original work.
while most known clustering strategies imported directly from the microarray analysis field were not.

Although this kind of analysis certainly does not provide a proof, the above results indicate that the theoretical framework is adequate for enumeration-based data, as expected. Additional examples and discussions can be found on the project’s website [7].

Conclusions

We developed a software tool, called Simcluster, for clustering libraries of enumeration-based data. It is important to note that Simcluster is built in accordance with a well-established mathematical framework for compositional data analysis, which provides principled procedures for dealing with the simplex space, and is thus applicable in contexts other than transcript enumeration.

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Figure 1: Screenshot of an analysis session using Simcluster’s web-based interface. Simcluster’s on-line version was designed to be a user-friendly interface for the command-line version. The screenshot shown is an illustration of an interactive session using the example data provided.

Figure 2: Clustering analysis of the Affymetrix dataset. Data produced by the Innate Immunity Systems Biology project [19, 5] and available as Additional File. This data is a set of Affymetrix experiments of mouse macrophages stimulated by different Toll-like receptor agonists (LPS, PIC, CPG, R848, PAM) during a time-course (0, 20, 40, 60, 80 and 120 minutes). Method: Euclidean distance with complete linkage agglomerative hierarchical clustering.

Figure 3: Simcluster’s clustering of simulated data based on Affymetrix expression levels. Transcript enumeration data produced by the simulation of a virtual transcriptome according to the Affymetrix expression levels. Sample size $n = 100,000,000$. Method: Simcluster’s complete linkage agglomerative hierarchical clustering.

Figure 4: Clustering of simulated data using Euclidean distance. Transcript enumeration data produced by the simulation of a virtual transcriptome according to the Affymetrix expression levels. Sample size $n = 100,000,000$. Method: Euclidean distance with complete linkage agglomerative hierarchical clustering.

Figure 5: Clustering of simulated data using correlation distance. Transcript enumeration data produced by the simulation of a virtual transcriptome according to the Affymetrix expression levels. Sample size $n = 100,000,000$. Method: correlation-based distance with complete linkage agglomerative hierarchical clustering.

Figure 6: Clustering of simulated data using cosine distance. Transcript enumeration data produced by the simulation of a virtual transcriptome according to the Affymetrix expression levels. Sample size $n = 100,000,000$. Method: cosine distance with complete linkage agglomerative hierarchical clustering.
Figure 7: Clustering of GSE694 SAGE kidney data using Simcluster. SAGE transcript enumeration data for several kidney parts. Glom: Glomerulus, PCT: proximal convoluted tubes, PST: proximal straight tubule, MTAL: medullary thick ascending limb of Henle’s loop, CTAL: cortical thick ascending limb of Henle’s loop, CCD: cortical collecting duct, OMCD: outer medullary collecting duct, DCT: distal convoluted tubule. Colors represent the biologically meaningful groups stated in Chabardes-Garonne et al. [15]. Method: Simcluster’s complete linkage agglomerative hierarchical clustering.

Figure 8: Clustering of GSE694 SAGE kidney data using Euclidean distance. SAGE transcript enumeration data for several kidney parts. Colors represent the biologically meaningful groups stated in Chabardes-Garonne et al. [15]. Method: Euclidean distance with complete linkage agglomerative hierarchical clustering.

Figure 9: Clustering of GSE694 SAGE kidney data using correlation distance. SAGE transcript enumeration data for several kidney parts. Colors represent the biologically meaningful groups stated in Chabardes-Garonne et al. [15]. Method: correlation-based distance with complete linkage agglomerative hierarchical clustering.

Figure 10: Clustering of GSE694 SAGE kidney data using cosine distance. SAGE transcript enumeration data for several kidney parts. Colors represent the biologically meaningful groups stated in Chabardes-Garonne et al. [15]. Method: cosine distance with complete linkage agglomerative hierarchical clustering.
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