

Mes clusters ont-ils un sens ?

Quelques indices statistiques et biologiques pour nous aider ...

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DIGIT-BIO seminar @ Ecully



Outline

- 1 Introduction: Clustering and RNA-seq co-expression
- 2 Validating co-expression clusters: why and how?
 - Internal metrics
 - Stability metrics
 - External metrics
- 3 Let's try it out!
- 4 Wrapping up

Gene co-expression is...

- **Simultaneous expression**¹ or **co-transcription**² of several genes
- **Similarity**³ (correlation, mutual information, ...) **of expression patterns** over a range of different experiments⁴

Related to shared regulatory inputs,
functional pathways, and biological process(es)⁵
+ a tool to study genes without known or predicted function

¹<https://en.wiktionary.org/wiki/coexpression>

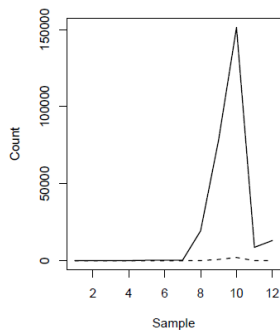
²<http://bioinfow.dep.usal.es/coexpression>

³<http://coxpresdb.jp/overview.shtml>

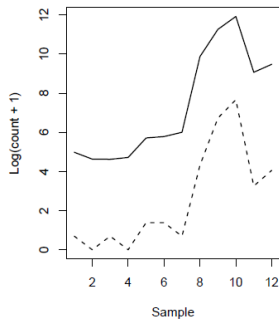
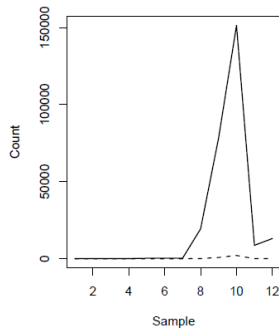
⁴Yeung *et al.* (2001)

⁵Eisen *et al.* (1998)

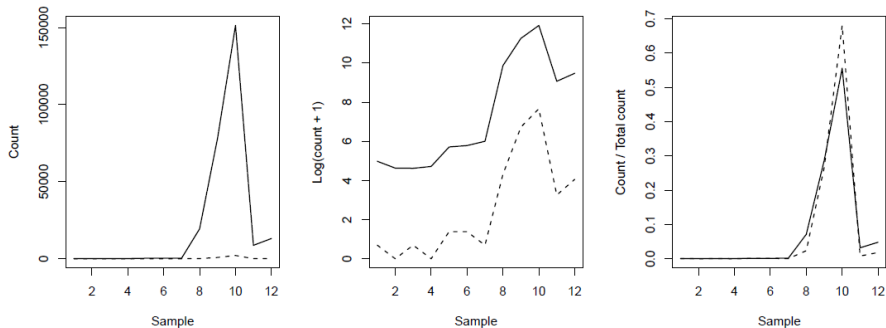
RNA-seq co-expression: counts, transformed counts, or profiles?



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- y_{ij} = raw count for gene i in sample j , with library size normalization factor s_j
- **Normalized profile for gene i :** $p_{ij} = \frac{y_{ij}/s_j}{\sum_{\ell} y_{i\ell}/s_j}$

Unsupervised classification (aka clustering)

Objective

Define **homogeneous** and **well-separated** groups of genes from transcriptomic data

What does it mean for a pair of genes to be **close**?
Given this, how do we define **groups**?

Two broad classes of methods typically used:

- 1 Centroid-based clustering (**K-means** and hierarchical clustering)
- 2 **Model-based clustering** (mixture models)

Model-based clustering for co-expression

Assume data \mathbf{y} come from K subpopulations, each modeled separately:

$$f(\mathbf{y}|K, \boldsymbol{\Psi}_K) = \prod_{i=1}^n \sum_{k=1}^K \pi_k f_k(\mathbf{y}_i; \boldsymbol{\theta}_k)$$

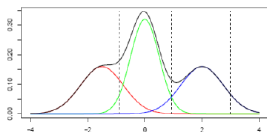
- $\boldsymbol{\pi} = (\pi_1, \dots, \pi_K)'$ are the mixing proportions, where $\sum_{k=1}^K \pi_k = 1$
- f_k are the densities of each of the components
- Microarrays: typically assume $\mathbf{y}_i|k \sim \text{MVN}(\boldsymbol{\mu}_k, \boldsymbol{\Sigma}_k)$
- RNA-seq: Poisson distribution for counts (HTSCluster), Gaussian distribution for **arcsine or CLR-transformed normalized profiles** (coseq)

→ Estimation (EM algorithm), model selection (BIC/ICL/slope heuristics), ...
 See DIGIT-BIO **Concepts en IA** talk by Cathy Maugis-Rabusseau for more!

From finite mixtures to clusters

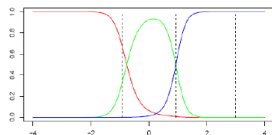
Distributions:

$$g(x) = \pi_1 f_1(x) + \pi_2 f_2(x) + \pi_3 f_3(x)$$



Conditional probabilities:

$$\tau_{ik} = \frac{\pi_k f_k(x_i)}{g(x_i)}$$



Maximum a posteriori (MAP) rule: Assign genes to the component with highest conditional probability τ_{ik} :

τ_{ik} (%)	$k = 1$	$k = 2$	$k = 3$
gene 1	65.8	34.2	0.0
gene 2	0.7	47.8	51.5
gene 3	0.0	0.0	100
...

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Why validate co-expression clusters?

- Avoid finding patterns in noise: does non-random structure actually exist in the data?
- Evaluate fit of clustering on data, compare different algorithms or results ⇒ Internal
- Determine “correct” number of clusters
- Determine robustness of clustering results ⇒ Stability
- Compare clusters to externally known labels
- Characterize clusters with respect to externally known information ⇒ External

Internal cluster validation

Evaluate goodness of clustering structure using data alone \Rightarrow similarity of genes within cluster vs distinctness of genes between clusters

- **Compactness**, or within-cluster variation
- **Connectivity**, or extent to which genes clustered together are also neighbors in the data space
- **Separation** between clusters



Image: 10.1093/bioinformatics/bti517

Examples of internal validation metrics

- **Silhouette statistic**: measure of how closely data within a cluster is matched (compactness) and how loosely it is matched to neighboring clusters (separation)

$$a(i) = \frac{1}{|C_I| - 1} \sum_{j \in C_I, i \neq j} d(i, j) \text{ and } b(i) = \min_{J \neq I} \frac{1}{|C_J|} \sum_{j \in C_J} d(i, j), \text{ for } i \in C_I$$

$$s(i) = \frac{b(i) - a(i)}{\max\{a(i), b(i)\}}, \quad -1 \leq s(i) \leq 1$$

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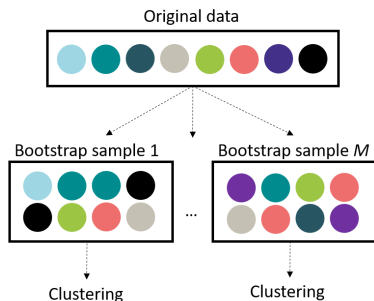
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- **Dunn index**: ratio of smallest distance between clusters and their diameter (largest intra-cluster distance)

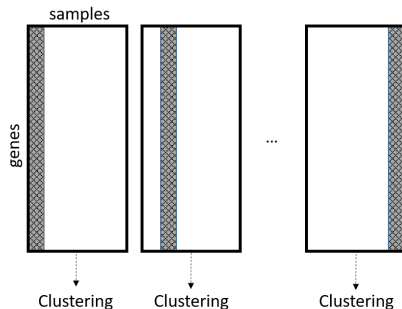
$$DI = \frac{\min_{C_I, C_J, I \neq J} \left(\min_{i \in C_I, j \in C_J} d(i, j) \right)}{\max_{C_M} \text{diam}(C_M)}$$

where $\text{diam}(C_M)$ is maximum distance among $i \in C_M$, $0 < DI$ (**maximize**)

Validation of cluster stability



- `fpc`: resampling of data using bootstrap → calculate Jaccard similarities to original clusters



- `clValid`: remove columns one by one, compare to clustering from full data (several criteria proposed)

External cluster validation

Evaluate ability of clustering algorithm to produce biologically meaningful clusters with respect to:

- Gene ontology (GO) term annotations
- A priori functional categorizations of genes
- Pathway membership
- ...

⇒ Assume set of F known biological classes (not necessarily disjoint)

Examples of external validation metrics: *comparison*

- **Adjusted Rand Index** (ARI): corrected-for-chance Rand index

$$R = \frac{TP + TN}{TP + FP + FN + TN} \in [0, 1]$$

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- **Biological Homogeneity Index** (BHI): if $B(i)$ is the known biological class of gene i ,

$$BHI = \frac{1}{K} \sum_{k=1}^K \frac{1}{n_k(n_k - 1)} \sum_{i \neq j \in C_k} I(B(i) = B(j)) \in [0, 1]$$

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- **Biological Stability Index** (BSI):

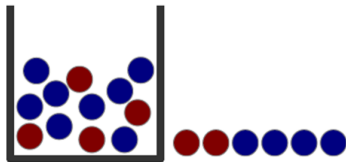
$$BSI = \frac{1}{F} \sum_{k=1}^F \frac{1}{|B_k|(|B_k| - 1)M} \sum_{\ell=1}^M \sum_{i \neq j \in B_k} \frac{|C^{i,0} \cap C^{j,\ell}|}{|C^{i,0}|} \in [0, 1]$$

based on removing each of the M data columns one at a time

Examples of external validation metrics: *characterization*

What biological processes are over-represented in each cluster?

- Gene Ontology (GO) terms = group genes into categories by a common biological property
- Assumptions: under the null hypothesis, genes are independent and equally likely to be grouped together in the list of interest (= cluster)
- Test for over-representation using a hypergeometric distribution (Fisher's exact test)



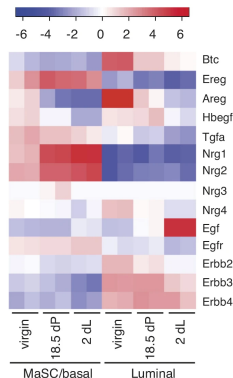
	Red	Blue	Total
Chosen	2	4	6
Remaining	4	8	12
Total	6	12	18

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Data description: Fu *et al.* (2015)

- RNA-seq experiment to study lineage of luminal cells in mouse mammary gland and changes in expression upon pregnancy & lactation
- **2 cell types** {basal stem-cell enriched cells, committed luminal cells} \times **3 statuses** {virgin, pregnant, lactating} \times **2 biological replicates**
 - Illumina HiSeq \rightarrow 30 million 100bp SE reads
 - *Pre-processing*: Subread to align reads to mm10 genome + featureCounts for quantification of Entrez genes (RefSeq)
 - *Initial analysis*: filter genes with weak expression or unambiguous/missing IDs + DESeq2 normalization/differential analysis



Supp Fig 4: 10.1038/ncb3117

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Validating clustering approaches in practice

Clustering results can be evaluated based on **internal and stability** criteria (e.g., statistical properties of clusters) or **external** criteria (e.g., functional annotations)

- Preprocessing steps will affect clustering outcome
- Methods that give different results depending on the initialization should be rerun multiple times to check for stability
- Repeated subsampling to identify consensus clusters (ConsensusClusterPlus)
- Most methods will find clusters even when no structure is present \Rightarrow good idea to compare to results with randomized data

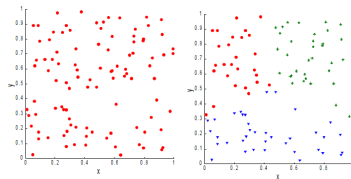


Image: Rumong Jin, *Cluster validation*

Final thoughts⁶

“

There is no single best criterion for obtaining a partition because no precise and workable definition of *cluster* exists. Clusters can be of any arbitrary shapes and sizes in a multidimensional pattern space. Each clustering criterion imposes a certain structure on the data, and if the data happen to conform to the requirements of a particular criterion, the true clusters are recovered.

”

⁶Jain & Dubes, 1988

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