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Scatterplot clustering for the integrative analysis of expression and methylation data

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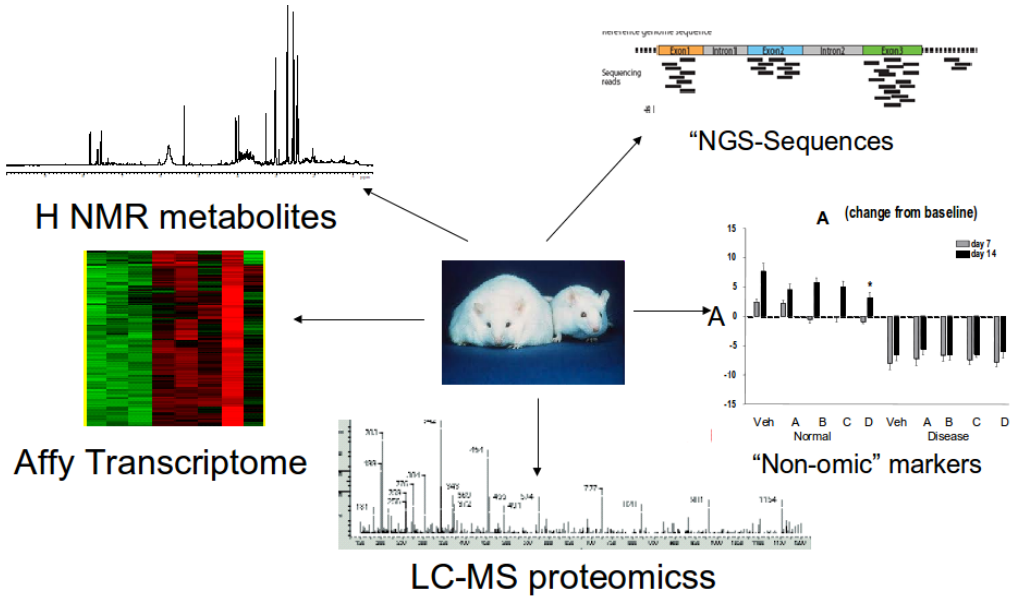
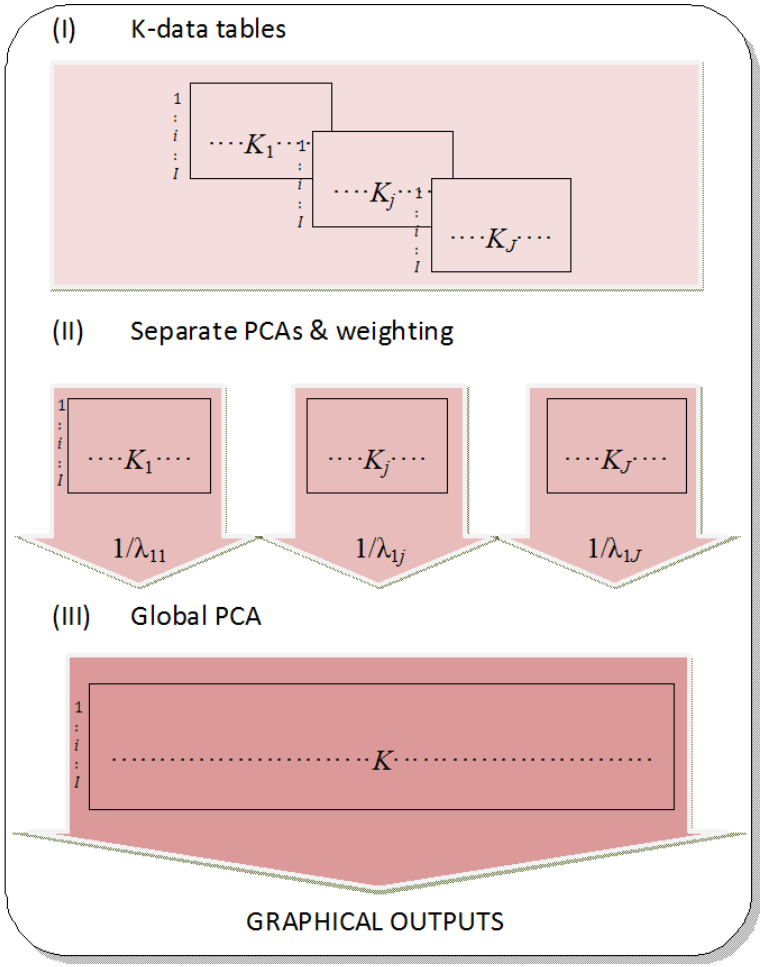
Post-genomics age: the next generation

- 'Omics' technologies are becoming increasingly important:
  - The advent of *next generation sequencing*, provides information on many type of *genomic*, *transcriptomic* or *epigenomic* data.
  - The generalization of high-throughput technologies allow to study biological processes at the different levels at which they happen.



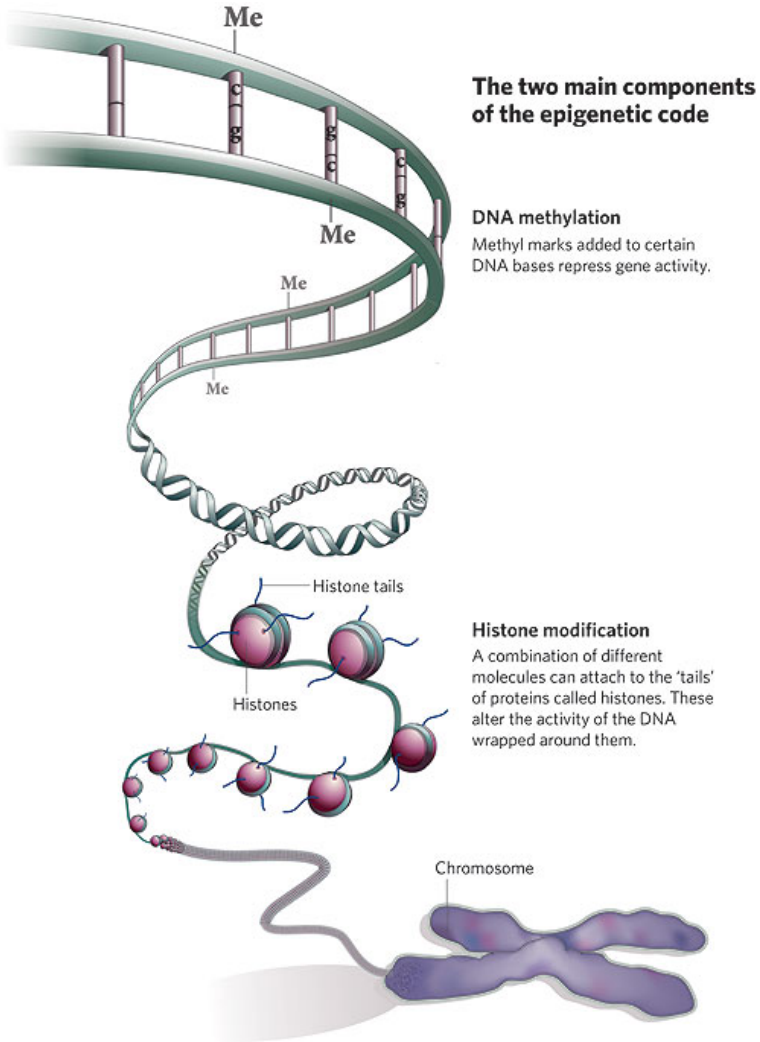
Data integration and systems biology -again

Statistics and bioinformatics are faced with the need for developing methods and tools for the integrative analysis of (big) data sets of different types and origins.



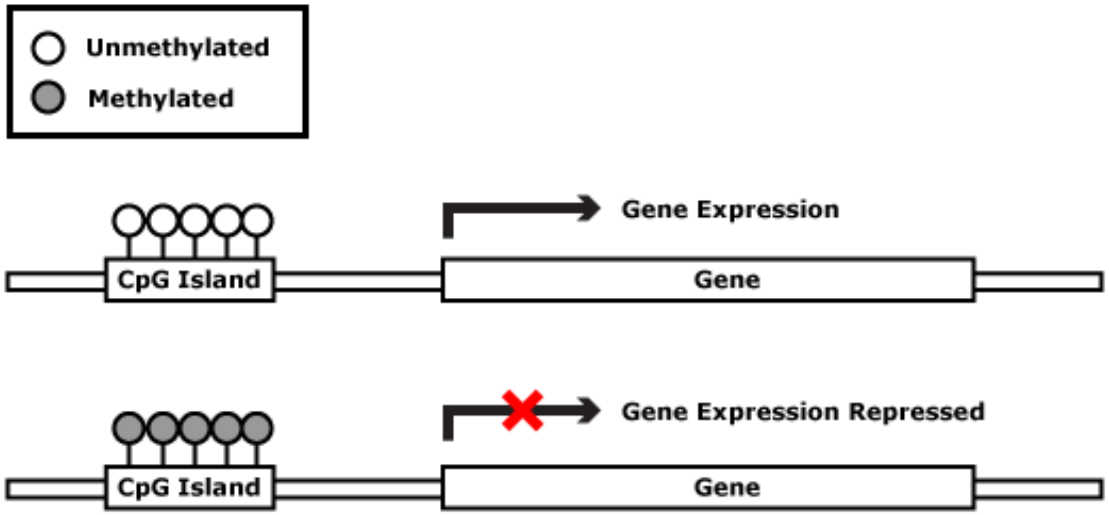
Epigenetics and epigenomics

- Epigenetics, *the study of environmental factors on gene expression in DNA*, is one of the disciplines that has experienced a renewed impetus:
- There is increasing evidence that many differentiation processes are triggered and maintained through epigenetic mechanisms such as *methylation* or *histone modifications*.



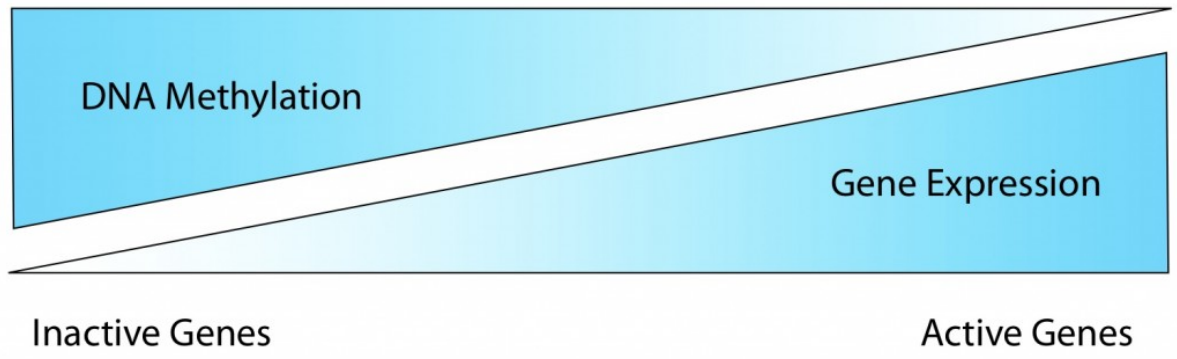
Methylation

- One main epigenetic regulatory mechanisms is methylation a process by which a gene's behavior is altered, but the gene itself isn't changed.
- Essentially methylation acts by inhibiting gene expression that is, the more methylated is a gene the more repressed is its expression



Methylation and gene expression

- Although the relation between methylation and gene expression is probably continuous ("*the more...the less...*"),

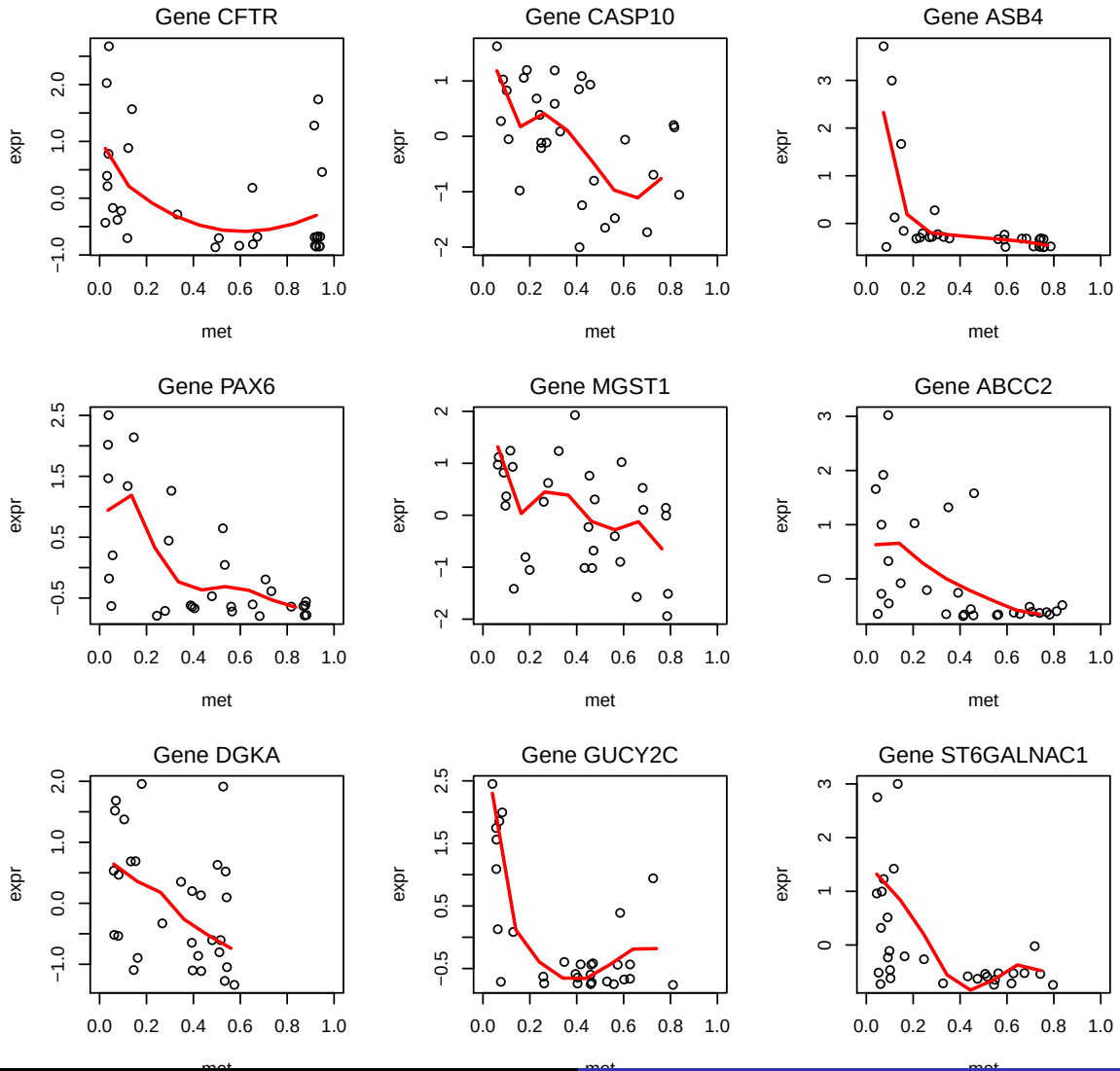


- methylation is, in practice, seen as a dual phenomenon
  - A methylated gene is "off"
  - An unmethlated gene is "on"
- Practical problem: **at which methylation level a gene is seen as "methylated" (that is, it is "turned off")?**

A colon cancer study

- This study originates in a work searching for colon cancer biomarkers.
- 30 cell lines characterized by increasing sensitivity to a drug were analyzed using several high-throughput methods: *transcriptomics*, *methylation*, *miRNAs*, *SNPs*, and *proteomics*.
- In this work we consider the problem of stablishing which genes were regulated by methylation.
- For each gene/methylation locus one has 30 points and a scatterplot showing the relation so we need methods to find patterns of scatterplots

Scatterplot patterns



Previous work

- Since measurements for methylation and expression are both continuous, scatterplots of these signals exhibit an L-shape pattern.
- Assuming that methylation is truly binary, there are two implications:
  1. the reflection point of the L-shape is an appropriate choice to binarize methylation data, and
  2. conditioning on the binarized on-off methylation status, the continuous valued methylation data and expression data should be independent,
- This motivates Liu(2012) to quantify the L-shape pattern using conditional mutual information (MI).

Objectives

- Study how gene expression is regulated by methylation in a set of colon cancer cell lines.
- Set up a method to detect the level of methylation at which a gene can be considered regulated by methylation (to be "on").
- Compare this method with other existing that have been developed to
  - detect methylation thresholds
  - detect patterns in scatterplots

Conditional Mutual Information

When studying methylation we are faced with two main questions:

- Which genes exhibit an L-shape, and
- what is the optimal threshold for binarizing methylation data for each L-shape gene.

The key

To determine whether methylation and expression of a gene exhibit an L-shape, compute the conditional Mutual Information (MI) for different choices of threshold to binarize the methylation data.

- If we consider the continuous valued methylation and expression data as two random variables X and Y, and denote a nominal threshold as t, the conditional MI can be written as a weighted sum of MIs on the two sides of the threshold.

$$cMI(t) = I(X, Y|X > t)P(X > t) + I(X, Y|X \leq t)P(X \leq t)$$

- When t is 0 or 1, cMI equals to the mutual information derived from all data points.
- For an L-shape gene, as t moves from 0 to 1, $cMI(t)$ first decreases and then increases, and its value approaches zero when t coincides with the reflection point.

Optimal threshold

Therefore,

Optimal threshold

The ratio $r = \frac{\min\{cMI(t)\}}{cMI(0)}$ for an L-shape gene is small, and $t^* = \operatorname{argmin}\{cMI(t)\}$ is the optimal threshold for dichotomizing the methylation data of this gene.

Joint distribution estimator

- To estimate the MI terms we use a kernel-based estimator, which
  - constructs a joint probability distribution by applying a Gaussian kernel to each data point,
  - and estimates the MI based on the joint distribution.
- The estimator is as follows:

$$I(X,Y) = \frac{1}{M}\sum_{i=1}^{M} \log \frac{M \sum_{j=1}^{M} e^{-\frac{1}{2h^2}((x_i-x_j)^2+(y_i-y_j)^2)}}{\sum_{j=1}^{M} e^{-\frac{1}{2h^2}(x_i-x_j)^2} \sum_{j=1}^{M} e^{-\frac{1}{2h^2}(y_i-y_j)^2}}$$

where h is a tuning parameter for the kernel width and empirically set $h = 0.3$.

Clustering using Spline regression

We implemented regression based on B-splines because they are particularly efficient due to the block-diagonal basis matrices that result.
Let

- $\varsigma = \{t_1 < \cdots < t_N\}$ non decreasing knot sequence
- $[t_m, t_{m+1})$ half open interval
- B_{mp} p-th order polynomial (degree $p-1$) with finite support over the interval and 0 everywhere else so that $\sum_{m=1}^{N-p} B_{mp}(x) = 1$
- then $s(x) = \sum_{m=1}^{N-p} B_{mp}(x) c_m$

Clustering using Spline regression (2)

To represent the curve we set:

$$y_{ij} = s(x_{ij})$$

So

$$\mathbf{y}_i = \mathbf{B}_i \mathbf{c}$$

with

- $\mathbf{B}_i = [B_{1p}\mathbf{x}_i, B_{2p}\mathbf{x}_i, \ldots, B_{Lp}\mathbf{x}_i]$ the spline basis matrix
- $\mathbf{c}$ the vector of spline coefficients.

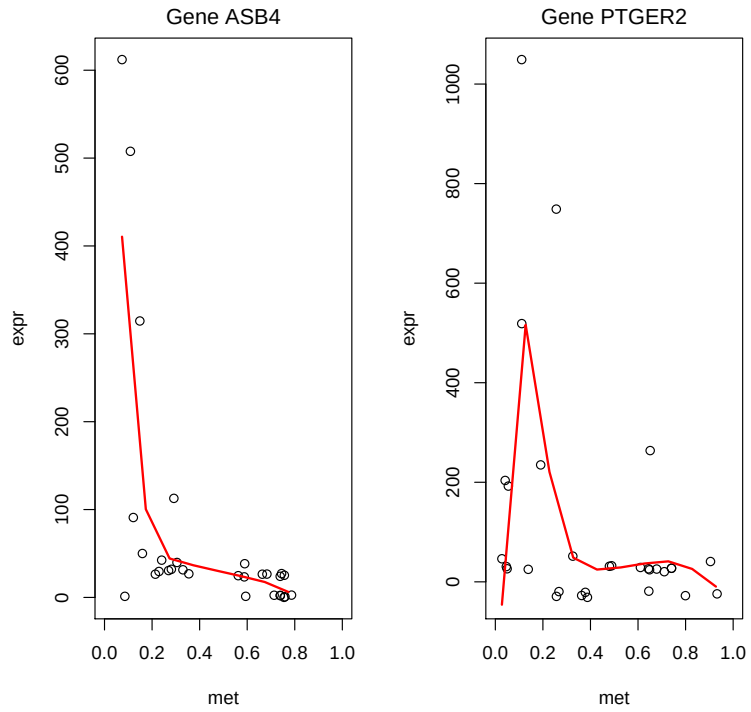
Clustering using Spline regression (3)

Algorithm

1. Selection of the genes with a negative significant correlation
2. Fit cubic regression splines
3. Data to cluster: splines coefficients
4. Calculation of a distance matrix between genes as $1 - \rho$
5. Hierarchical clustering

Results (1) Splines–based regression

- After the previous selection of genes we worked with 191 genes
- We decided to choose 5 clusters
- The 2 first clusters included the genes with an L-shape



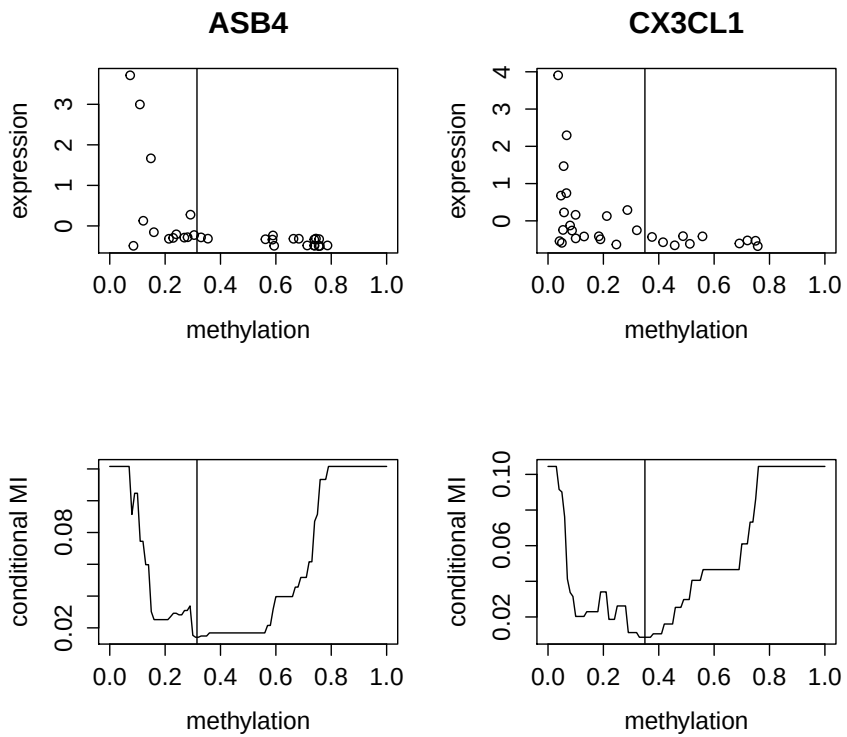
Results (2) Conditional Mutual Information

- No previous selection of the genes was needed
- We filtered for L-shapes using a combination of three criteria:
  - the ratio $r < 0.25$
  - unconditioned MI $cMI(0) > 0.1$
  - the median expression on the left side of the optimal threshold t^* is higher than the median expression on the right side.
- The parameters are chosen according to a random permutation test (see Liu(2012)).
- According to the above criteria, a total of 641 genes are selected to be L-shape genes.

Results (3) Comparison between the methods

The results of both methods that can be summarized in the following table:

Initial selection	191	641
Cluster	Splines	cMI
1	140	102
2	22	16
Total	162	118



Conclusions

- We have found similar results between both methods.
- Biological interpretation is still being done by biological researchers although results are consistent with the hypothesis (we have found genes regulated by methylation).
- Sample size is a limiting factor: cMI works better with hundreds of samples but one may have a very small number (real cases: 30, 12)

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