Nonnegative Matrix Factorization for analysis of metabolic pathways associated to fiber digestion from metagenomics data.

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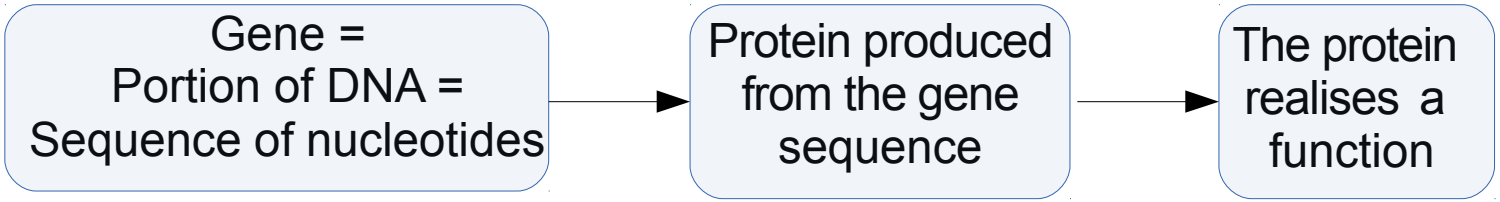

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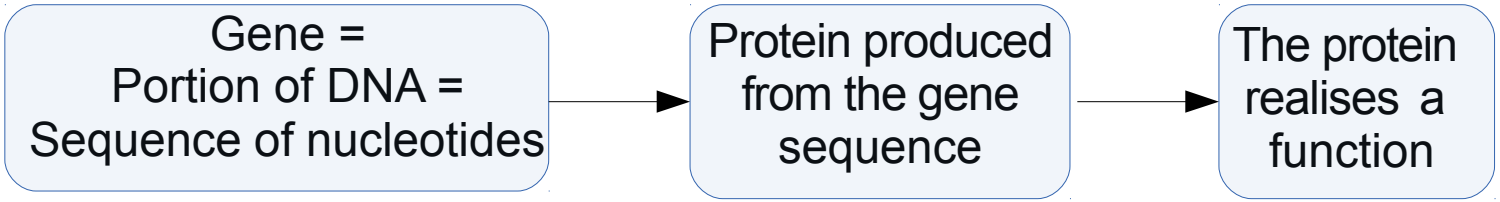

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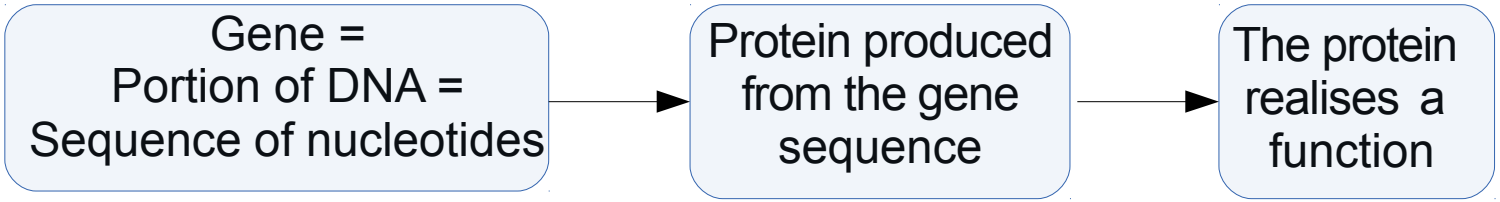

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- **Genomics and metagenomics**
  Genomics : analysis of the genome of a given organism (animal or plant)
  Metagenomics : analysis of genes in a given environment sample
  ↪ genes are not gathered by organisms.

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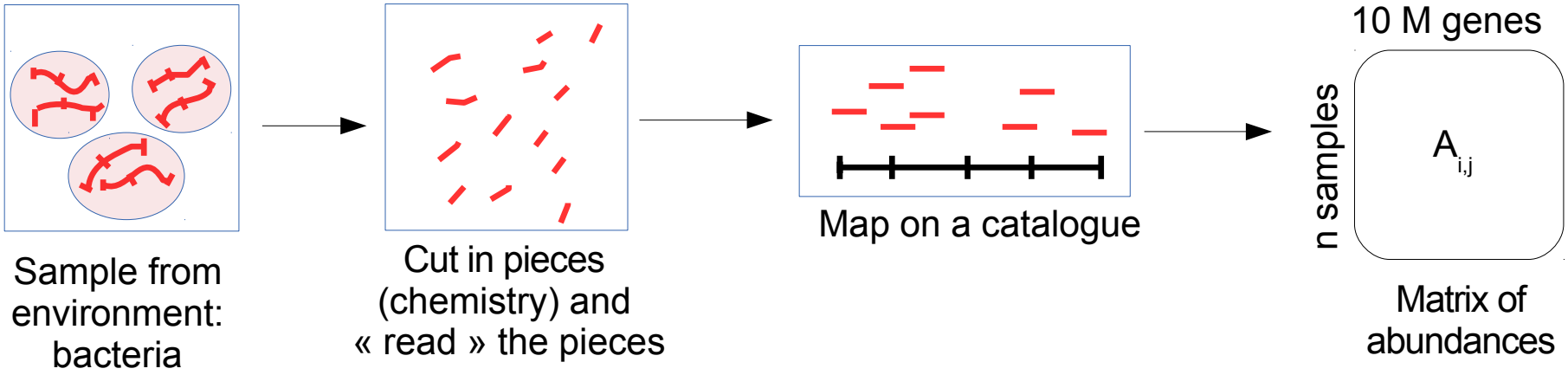

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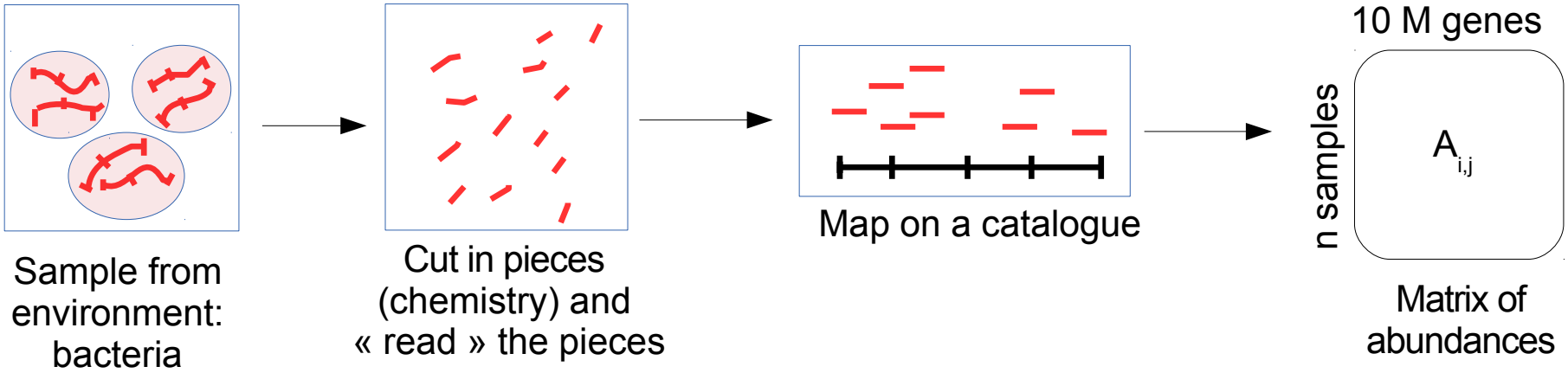

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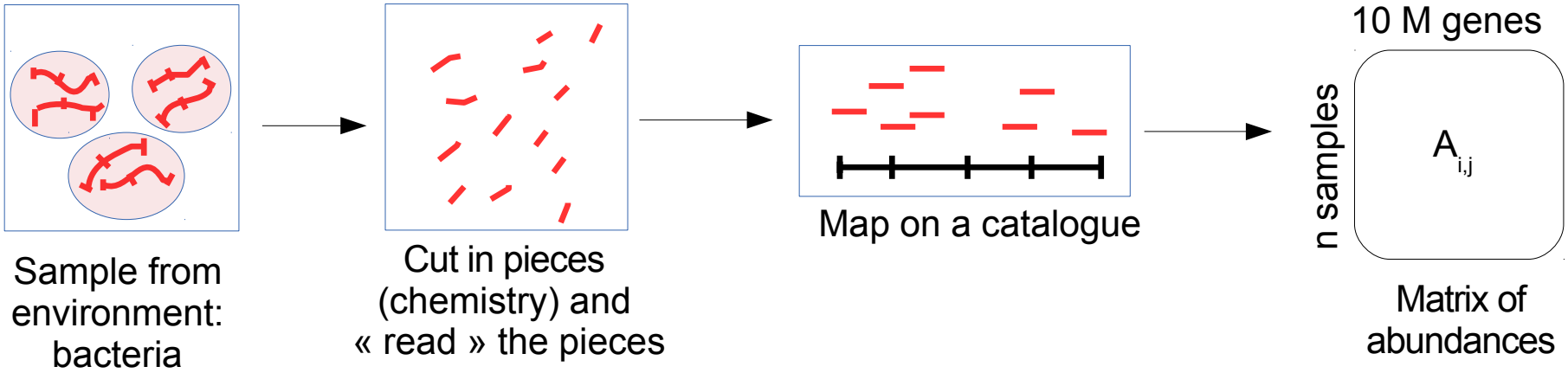

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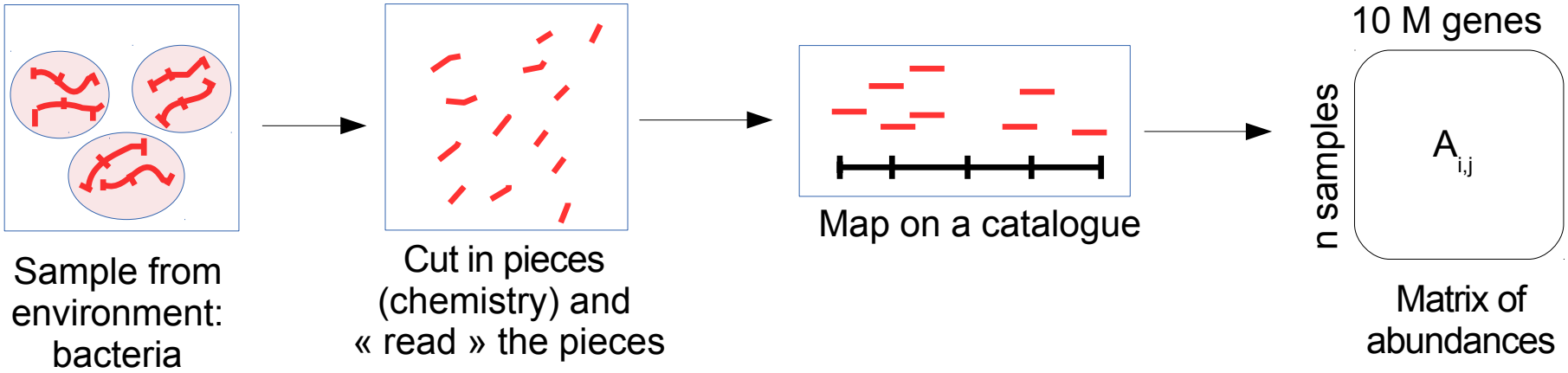

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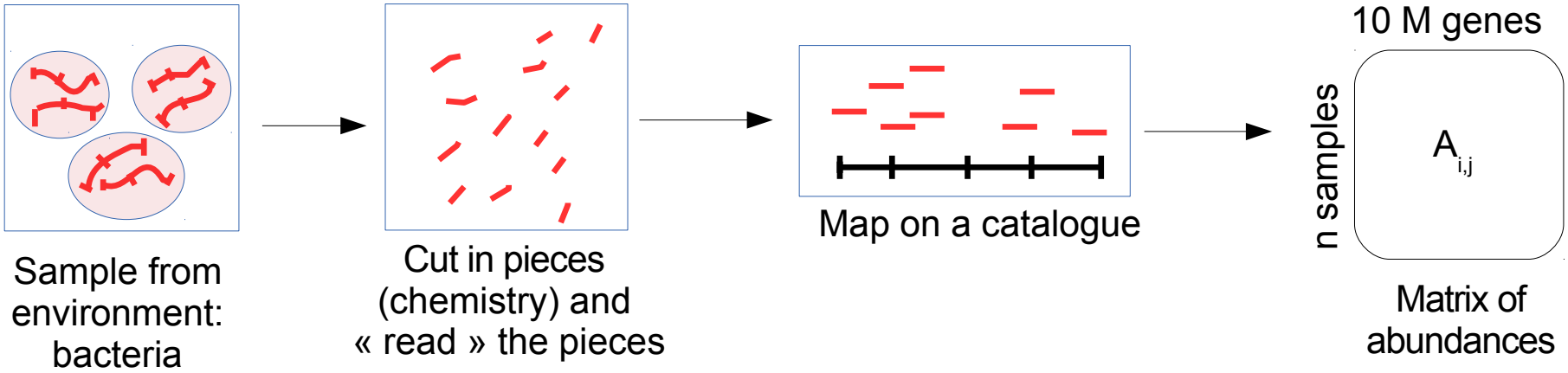

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  - $\hookrightarrow$ Groups of gene with similar functions from biological knowledge / sequence of translated proteins

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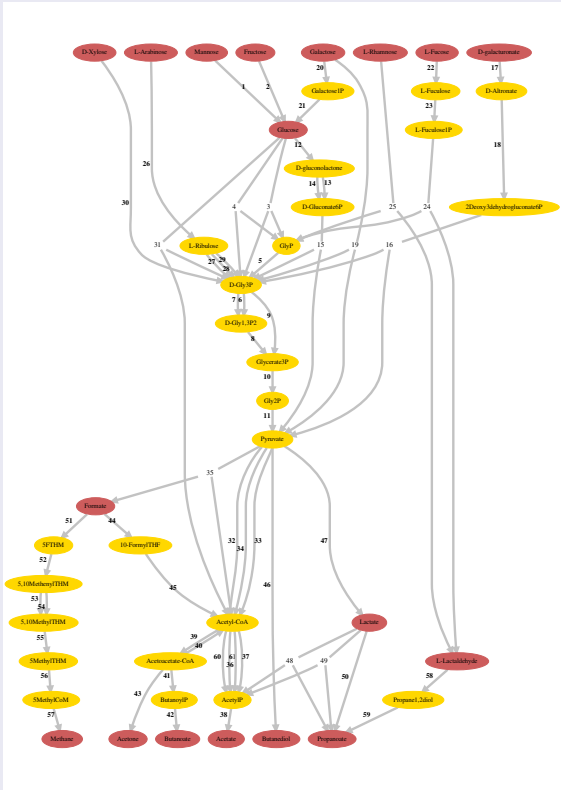
Fiber digestion metabolism

- 86 elementary reactions extracted from literature
- nodes = chemical

  arrows= reactions



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  ↪ For some elementary reactions, KEGG provides a list of bacterial genes which can realise this reaction.

  ↪ list of bacterial genes $\Leftrightarrow$ list of columns in the abundance matrix
- Therefore, from a metagene abundance matrix $A = n \times 10^7$, we can compute the **abundance of reaction r in sample i** as :

$$\sum_{\text{genes } g \text{ associated to reaction } r} A_{i,g}$$

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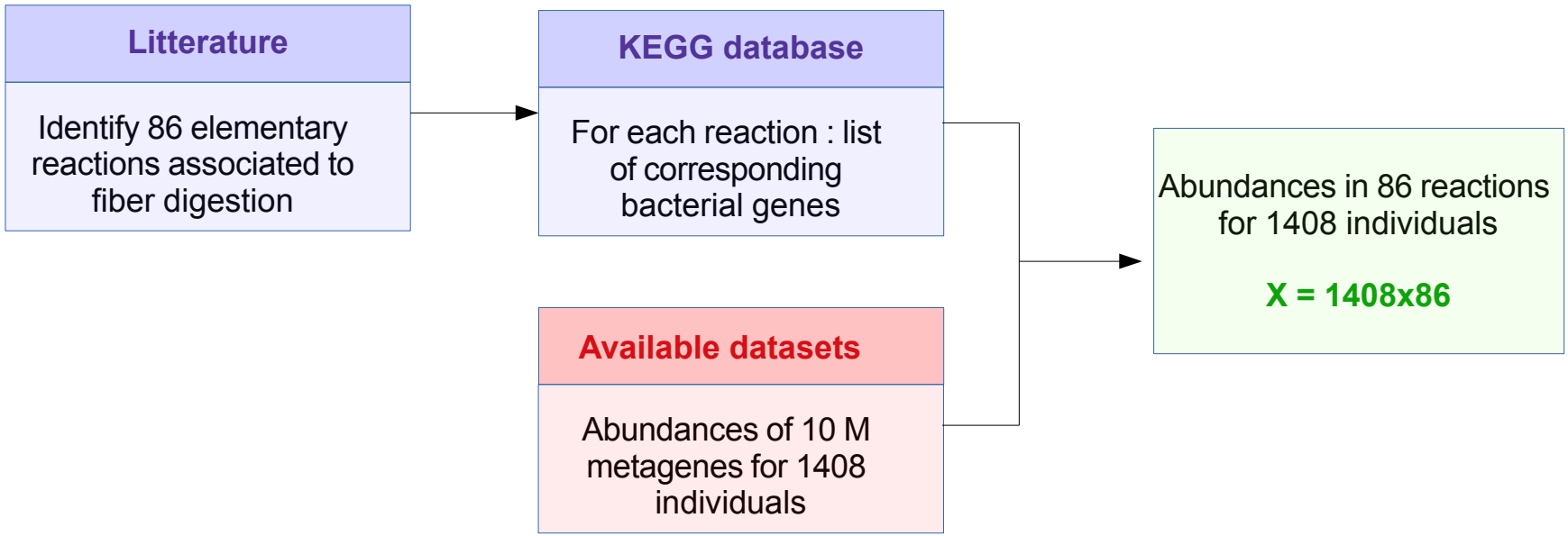

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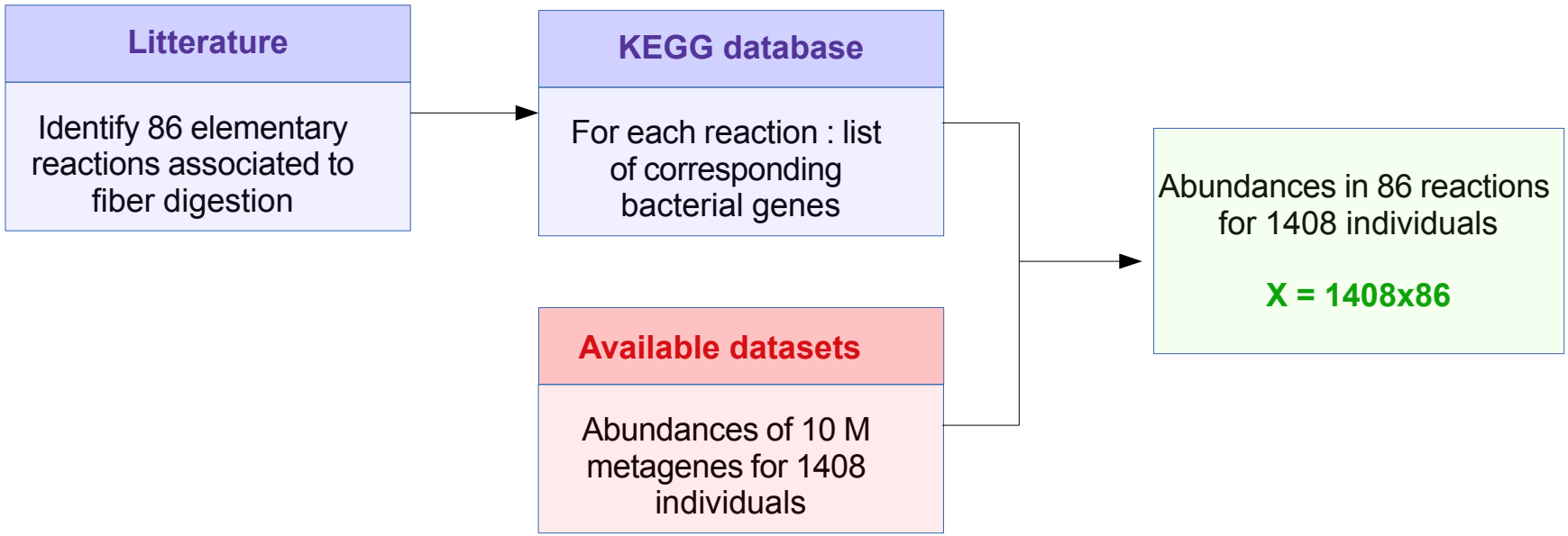

$\hookrightarrow$ $(X_{i,j})_{i=1,\dots,n,j=1,\dots,p}$: matrix with **abundances of p=86 elementary reactions** associated to fiber digestion, for **n=1408 individuals**.

Metabolic pathways

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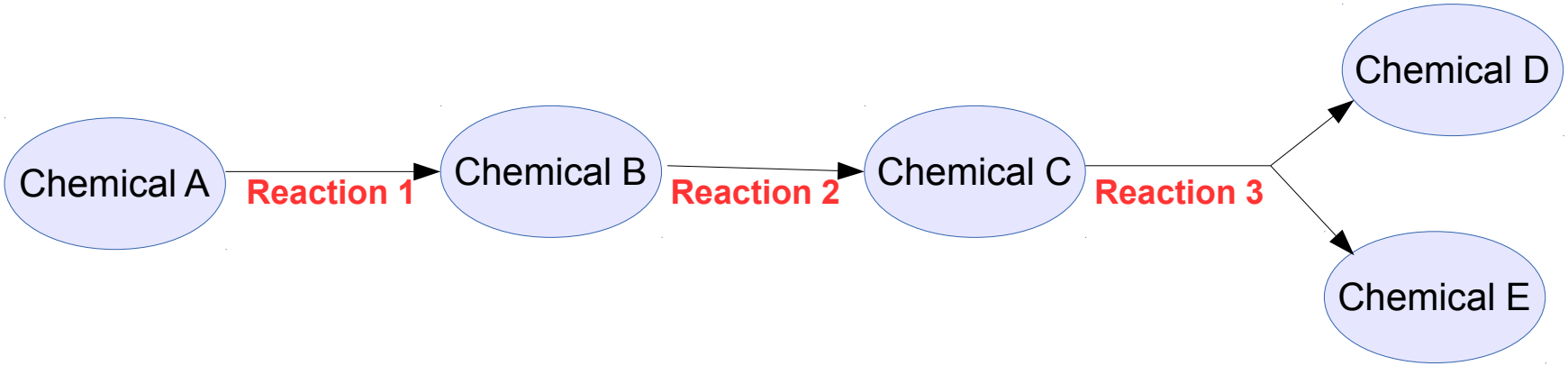

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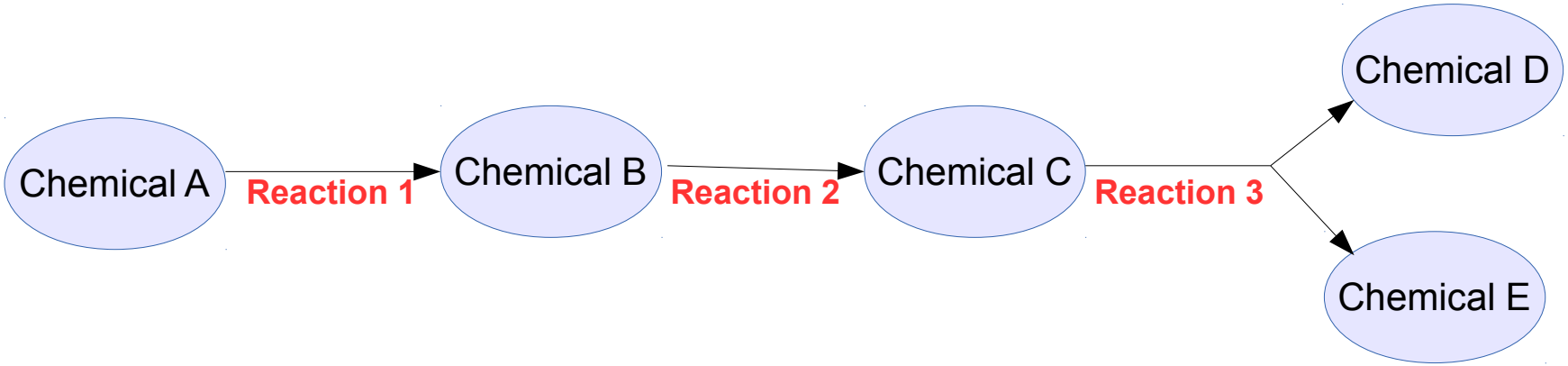

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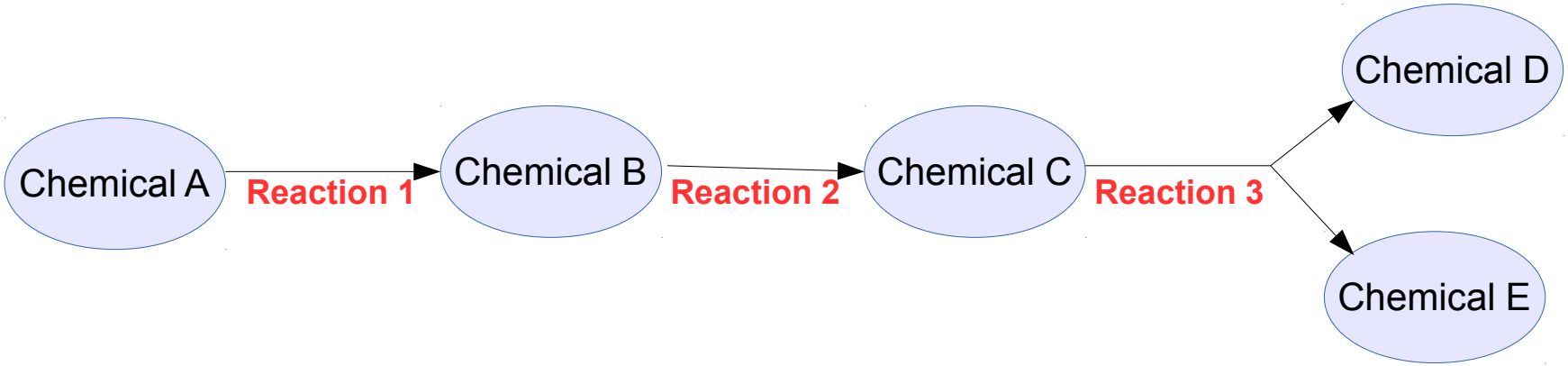

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- **Our goal** : extract the main metabolic pathways associated to fiber digestion from data matrix X.

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$\hookrightarrow$ **Nonnegative Matrix Factorization (NMF)**

- **Formulation of the NMF problem.** Let $X \in (\mathbb{R}^+)^{n \times p}$ be a matrix with non-negative coefficients. The NMF decomposition is the solution of the following minimisation problem :

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- **NMF in literature**
  - Originally developed in signal theory for source separation.
  - Then applications to genomics.
  - Methodological developments : mainly focused on algorithms

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- **Generalized Kullback-Leiber distance**

  $$D_{KL}(X|WH) = \sum_{i,j} \left(X_{i,j} \log \frac{X_{i,j}}{(WH)_{i,j}} - X_{i,j} + (WH)_{i,j} \right)$$

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- Rq : KL and Frobenius present computing facilities that can not be generalised.

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$\hookrightarrow$ For Frobenius and KL distances, we obtain additive and multiplicative update rules.

  - No theroretical guarantee of convergence.
  - No generalisation to other distance or additional constraints.

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$$\begin{cases} D(X, W_j H_{j-1}) + pen(W_j, H_{j-1}) \leqslant \mathsf{D}(X, W_{j-1} H_{j-1}) + pen(W_{j-1}, H_{j-1}) \\ D(X, W_j H_j) + pen(W_j, H_j) \leqslant \mathsf{D}(X, W_j H_{j-1}) + pen(W_j, H_{j-1}) \end{cases}$$

$\hookrightarrow$ For Frobenius and KL distances, we obtain additive and multiplicative update rules.

  - No theroretical guarantee of convergence.
  - No generalisation to other distance or additional constraints.
- We implemented alternate minimisation using general algorithm for constrainted convex optimisation.

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  - Concordance of W : for a individual profile matrix W, let S be the two-by-two correlation matrix between individual profiles W_i and $W_{i'}$: S is called the **similarity** matrix associted to W.
    Then the concordance index between two initialisations r and r' is defined as

$$1 - \sqrt{\sum_{i \neq j} \left(S_{i,j}^{(r)} - S_{i,j}^{(r')} \right)^2} \quad (1)$$

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  - Compute NMF with $X[I_1,]$ and $X[I_2,]$: (W_1, H_1), (W_2, H_2).
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- **Bi-cross-validation** : version of CV adapted to NMF.

Recall : cross-validation in regression.

Let $X \in \mathbb{R}^{n*p}$, $Y \in \mathbb{R}^n$, and $\{\hat{f}_\lambda, \lambda \in \Lambda\}$ a set of estimating procedures of the regression function $r(x) = \mathbb{E}[Y|X = x]$.

Let $I_1 \sqcup I_2 \sqcup \cdots \sqcup I_N = \{1, \ldots, n\}$. For $\ell = 1, \ldots, N$,

- Compute $\{\hat{f}_\lambda^{(\ell)}, \lambda \in \Lambda\}$ from $X[-I_\ell,]$ and $Y[-I_\ell]$.
- For $i \in I_\ell$, $\hat{Y}_i^\lambda = \hat{f}_\lambda^{(\ell)}(X_i)$.
- CV-error : $E^{\ell,\lambda} = \sum_{i=1,\ldots,n}(Y_i - Y_i^\lambda)^2$

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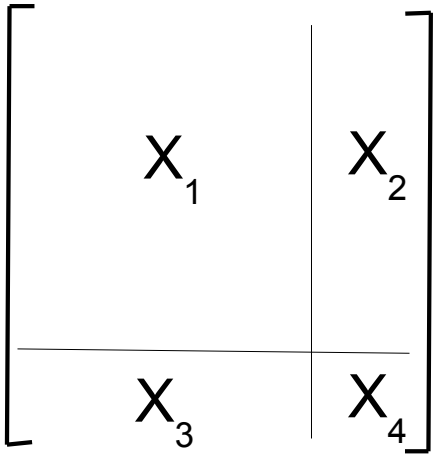
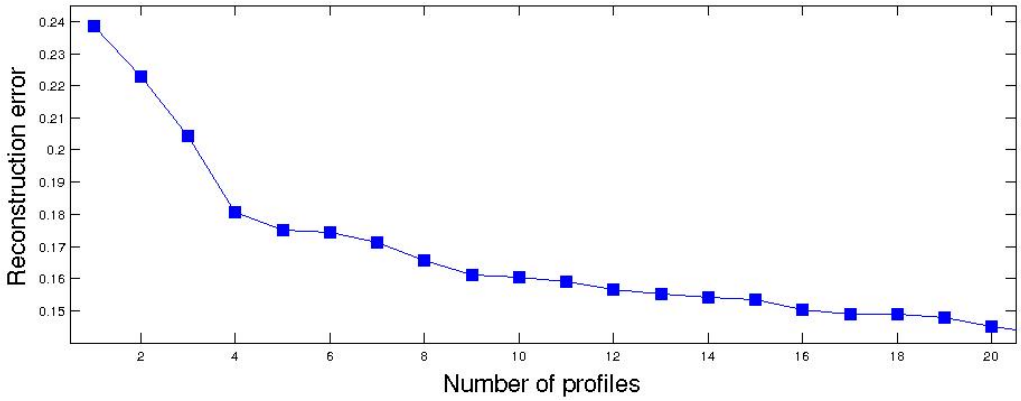
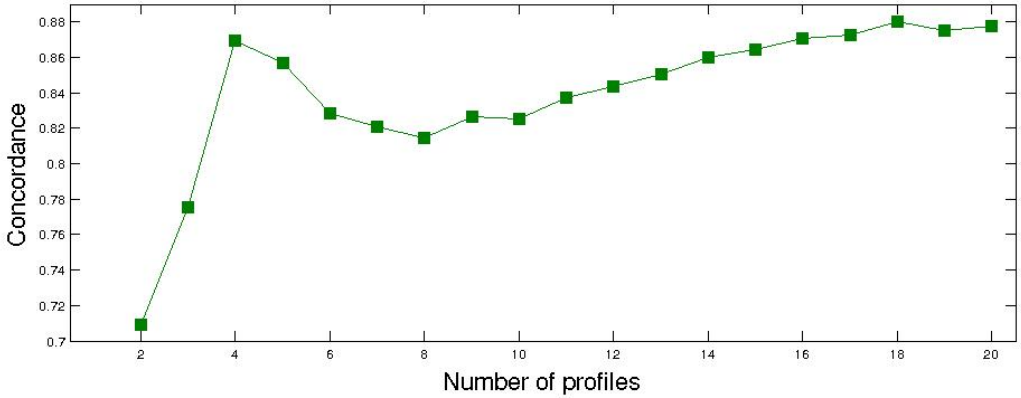
Bi-cross-validation in NMF

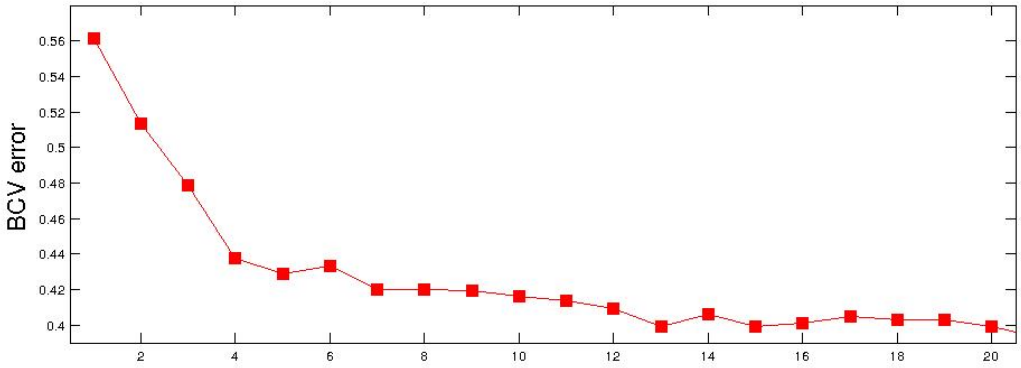


- $(W^{train}, H^{train}) = \arg\min D(X_1, WH) + pen(W, H)$
- $H^{val} = \arg\min D(X_2, W^{train}H) + pen(W^{train}, H)$
- $W^{val} = \arg\min D(X_3, WH^{train}) + pen(W, H^{train})$
- Bi-CV error : $D(X, W^{val}H^{val})$.

Results on our data







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- Similarly to regression :
  - L^1-penalty favor sparsity (possible lost of significant variables)
  - L^2-penalty usually offer a better reconstruction but no sparsity (less easy to interprete)
  - Elasticnet : trade-off
  - Group-lasso, etc : favor biological model of pathway/individual profiles

A few word about unicity of NMF decomposition

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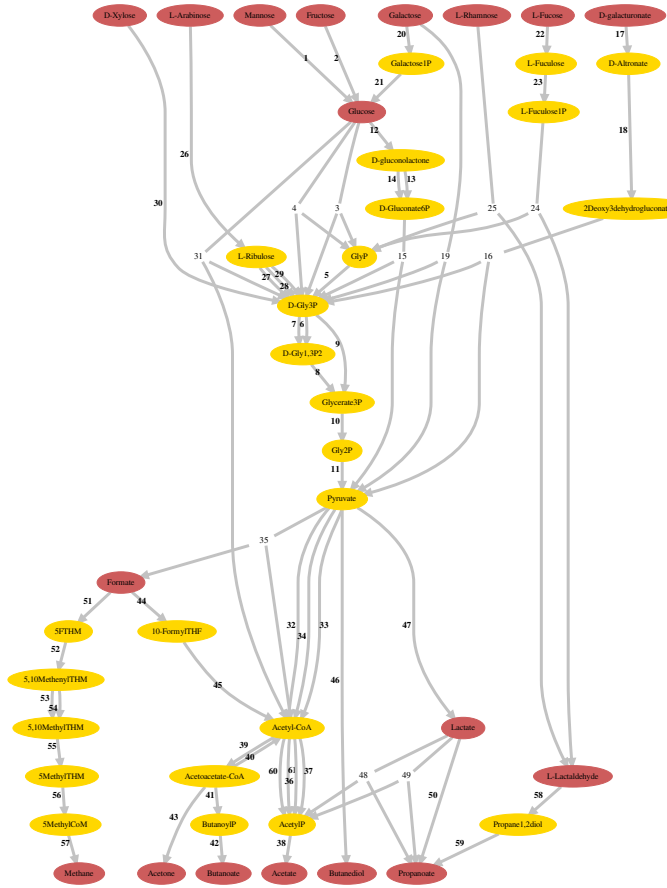
- In practise, we observe almost identical results up to scaling and permutations over repeated initialisations of the algorithm.

Inclusion of a priori knowledge

- **Graph of reactions involved in fiber digestion**
  - Arrow = elementary reaction
  - Node = chemical
  - Intra/extra cellular chemicals



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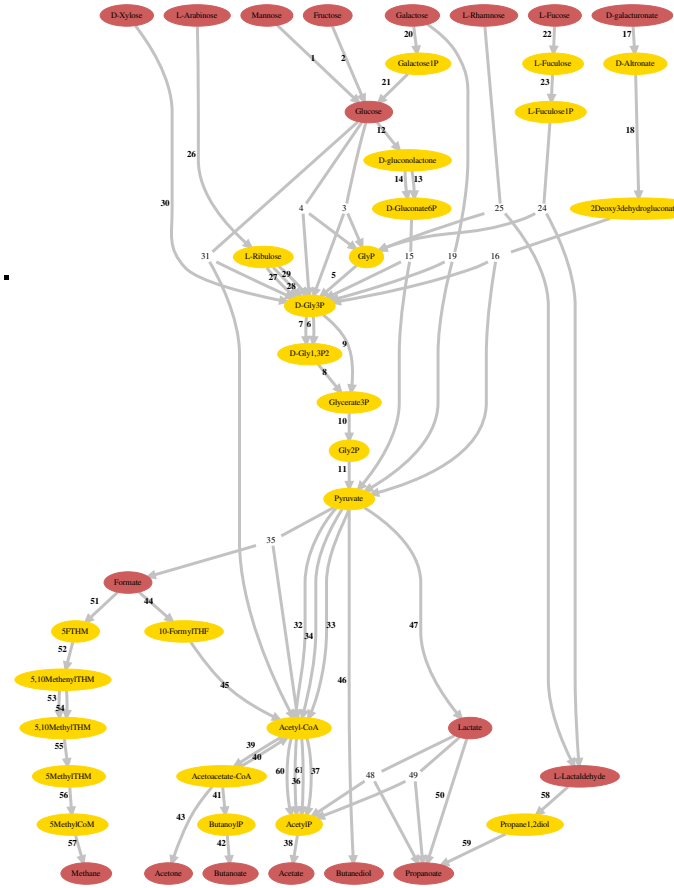

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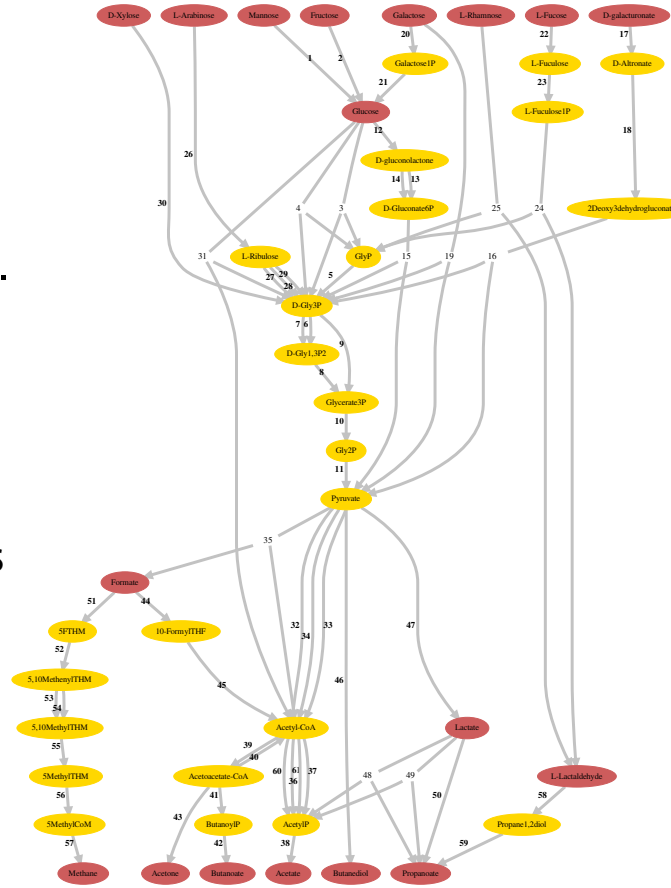

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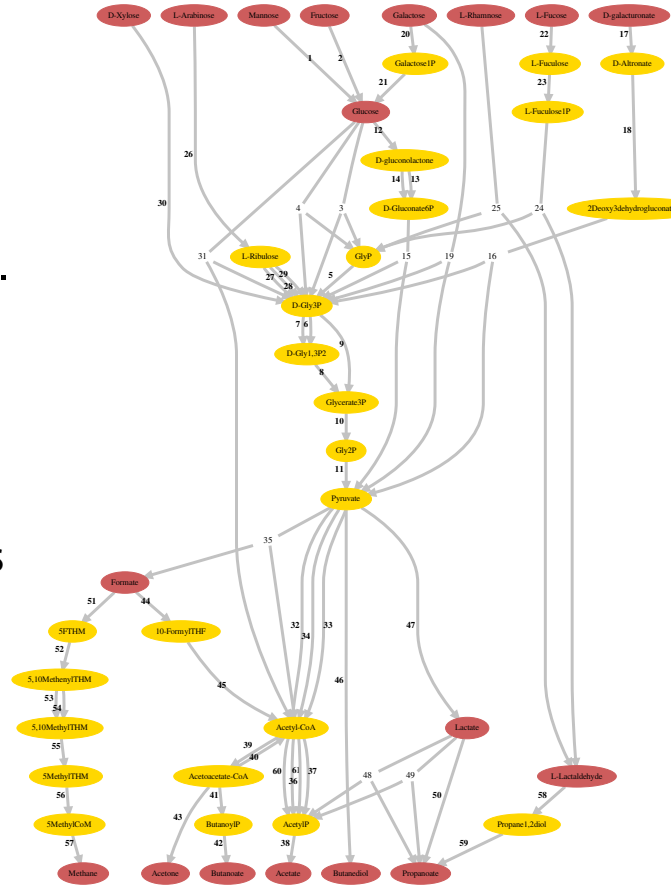
- We show that it's equivalent to a linear constraint :

$$\frac{1}{\delta}\mathbf{Q}^{-}\mathbf{H}^{\mathbf{t}} \leqslant \mathbf{Q}^{+}\mathbf{H}^{\mathbf{t}} \leqslant \delta\mathbf{Q}^{-}\mathbf{H}^{\mathbf{t}} \quad \text{with} \quad \delta > 1$$

with Q^+ and Q^- defined from the graph.



More precisely :

- Let Q be the adjacence matrix of the graph without extra-cellular chemical : for each reaction j and chemical c

$$Q_{c,j} = \begin{cases} 1 \text{ if reaction j reaches metabolite c} \\ -1 \text{ if reaction j originates on metabolite c} \\ 0 \text{ otherwise} \end{cases}$$

- Let $Q = Q^+ - Q^-$ the decomposition of Q in positive/negative part.
- For a metabolic pathway ℓ,
  $(Q^+H)_{\ell,c}$ = sum of proportion in reactions which reach metabolite c
  $(Q^-H)_{\ell,c}$ = sum of proportion in reactions which originate from metabolite c
- Thus, the proportion of reactions which create and degradate metabolite c have same order if :

$$\frac{1}{\delta}(Q^-H)_{\ell,c} \leqslant (Q^+H)_{\ell,c} \leqslant \delta(Q^-H)_{\ell,c} \quad \text{with} \quad \delta > 1$$

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- α is chosen by bi-cross-validation.

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- Our first goal is to prove that our whole method :
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- Our results are mainly descriptive.

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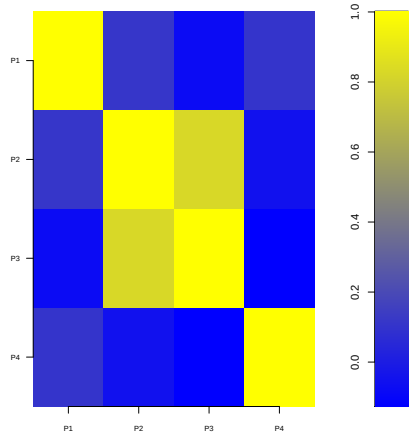

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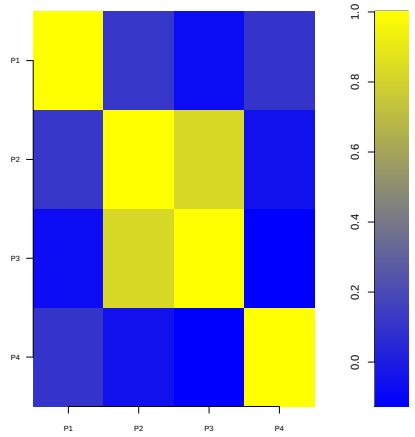

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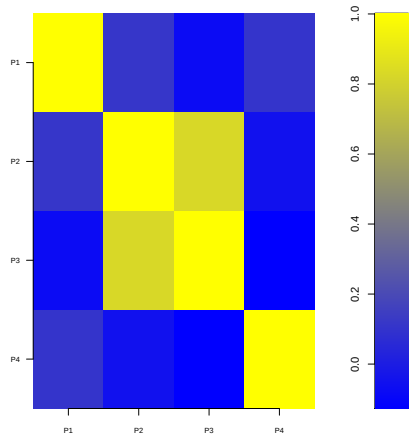

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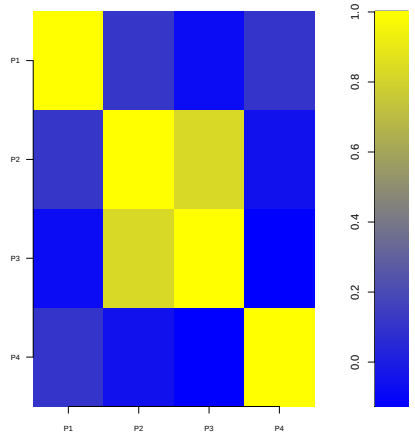

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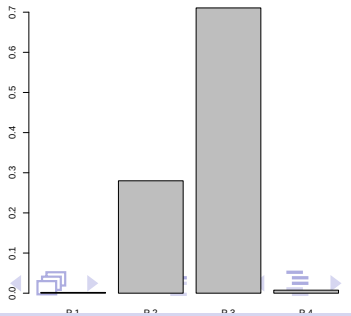
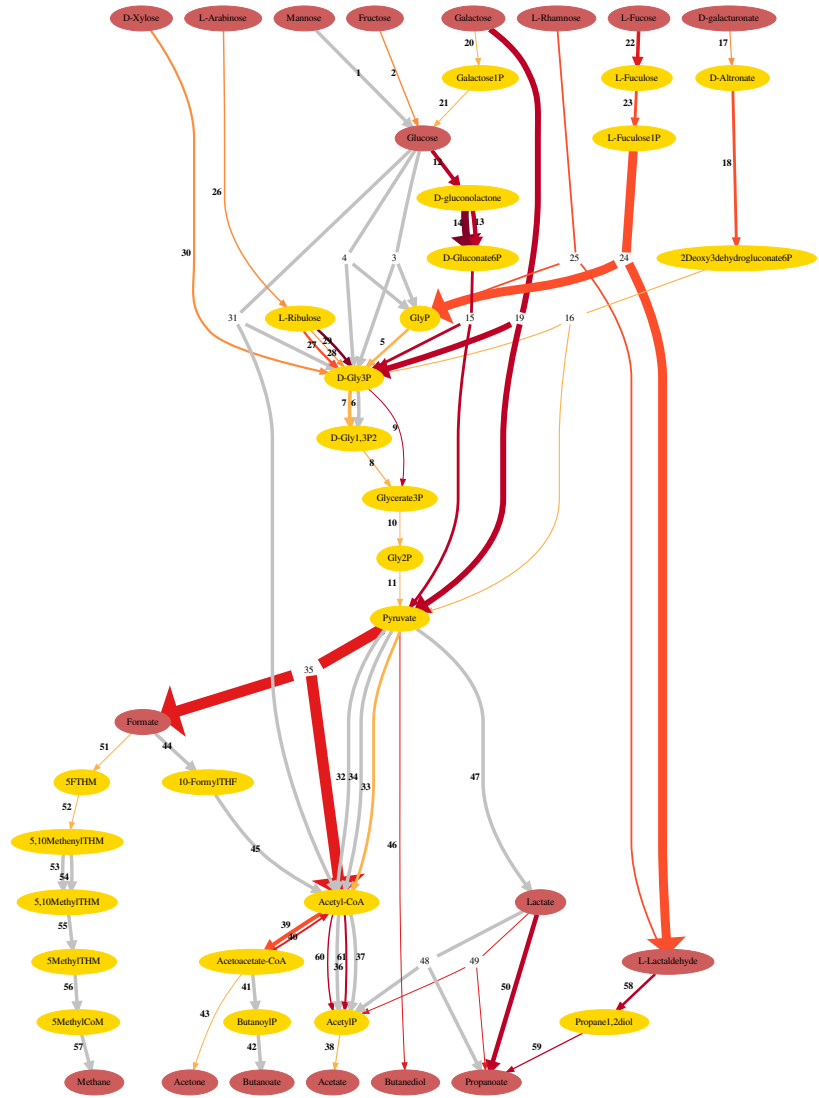


Analysis of H

Pathways in H are represented as weighted subgraphs

$\hookrightarrow$ Biological interpretation .

$\hookrightarrow$ Pathways with a strong total signal include more reactions.



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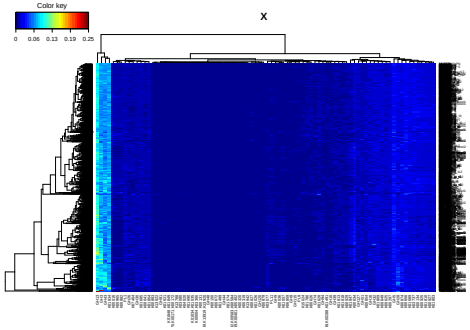

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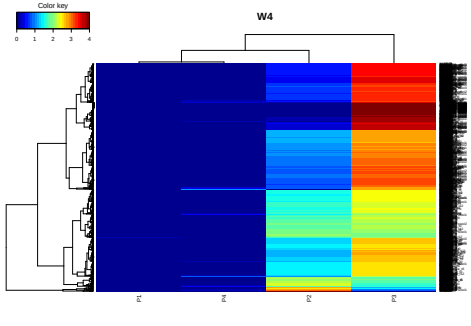

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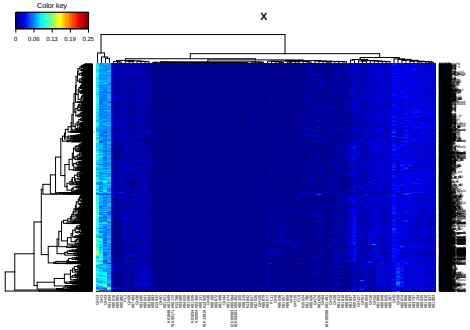

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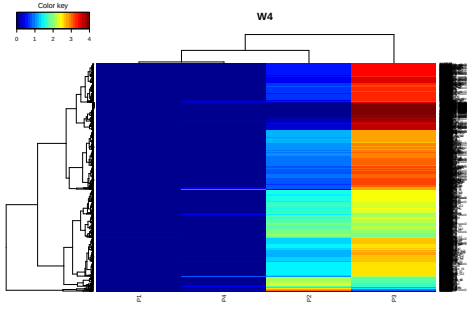

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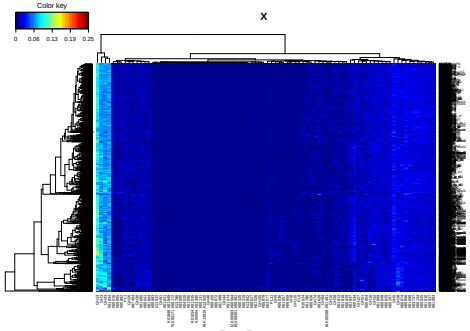

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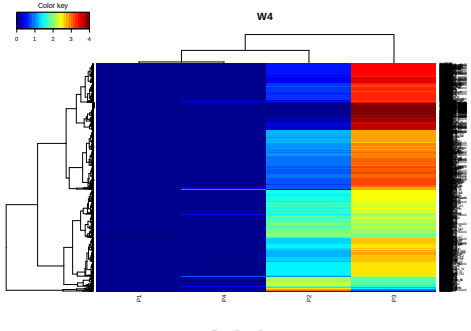

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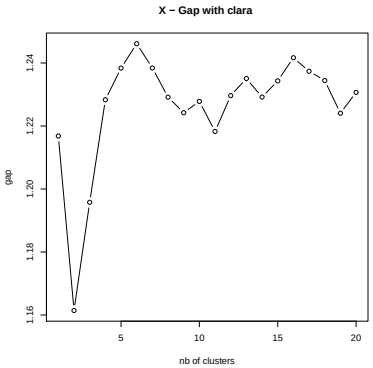
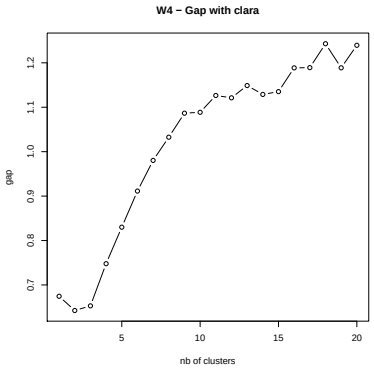


X

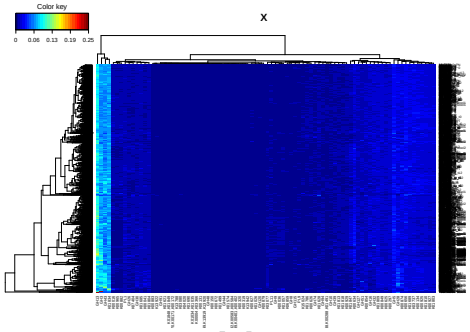


W

Analysis of the individual profiles in W (1)

Is there an underlying clustering structure in matrix W ?

- Visual examination : **hierarchical clustering** of rows and columns.



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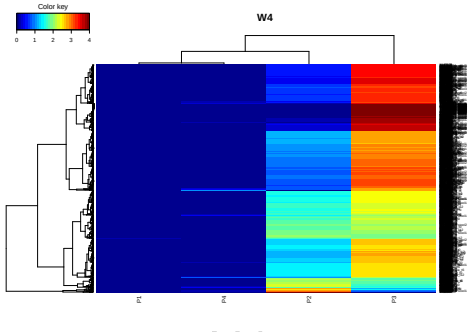
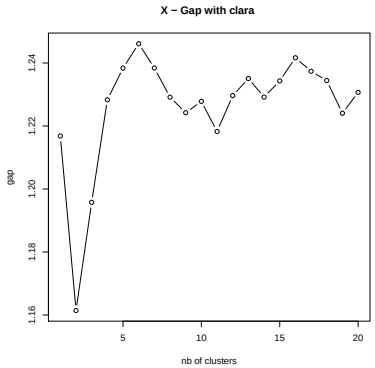


W

- Gap = difference between the **ratio "variance intra cluster / total variance"** between **true and bootstrapped** data
  - For a given clustering method, compute the gap for $k = 1, 2, ...$ clusters
  - Asa the gap decreases when a new cluster is added, this cluster is not significant.



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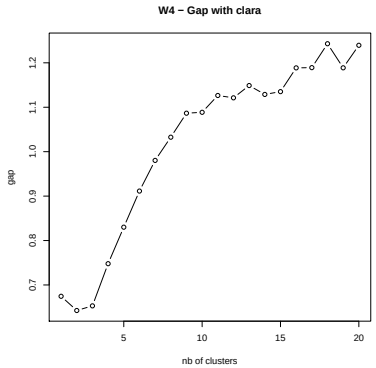

W

Conclusion :
- no strong clustering.
- smaller significant clusters in W.

Analysis of the individual profiles in W (2)

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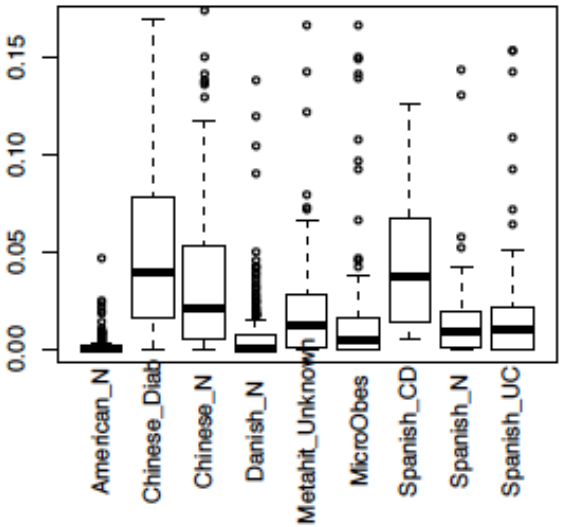
  $\hookrightarrow$ Caution in interpretation
- **Example of visual result** : boxplot of abundances of metabolic pathway 4 by study*disease :



- In Spanish study, pathway 4 is more present in Crohn disease patients.
- **Difficult to validate statistically**
  - Multiple testing problem.
  - Pathways have been built to discriminate individuals : p-values may be biased.

  $\hookrightarrow$ Perspective for validation on an independent data set of Crohn/healthy indivduals.

Summary of the biological analysis

- Goal : **explore potential of our methodology** to analyse metabolic pathways based on metagenomics data
  $\hookrightarrow$ Focus on fiber digestion in gut
- Procedure :
  - **Build a matrix** with abundances in reactions associated to fiber digestion, **combining metagenomic data and a priori knowlegde**.
  - **Decompose** this matrix in pathways by NMF
- Results
  - This procedure provides results easy to handle and interprete.
  - Some biological knowledge is recovered
  - NMF can enlight metabolic pathways which represent a low signal but may be significantly discriminative.
- Perspective : validation on independent data.

Summary on the NMF methodology

NMF procedure is defined by the following elements :

- Distance = statistical model
  $\hookrightarrow$ We have chosen Frobenius as a first approach.
- Criterion for the choice of the reduced dimension : linked to the global goal of NMF decomposition.
  $\hookrightarrow$ We have proposed more interpretable criteria than the usual numerical stability.
- Penalty : linked to general biological assumptions / posterior use of the NMF results
- Possibly : additional constraints
- Algorithm
  $\hookrightarrow$ We have developed an alternate minimisation algorithm that can be adapted to various distances and constraints

Methodological perspectives

- Compare results on our data with Frobenius and KL distances (adapt algorithm for KL distance)
- Develop a simulation framework for NMF
  $\hookrightarrow$ Further : pseudo-simulations which keep the correlation structure of experimental data and generate a controlled signal on them
- Propose an analysis of the criteria used for the choice of the reduced dimension k, at first using simulations.
  $\hookrightarrow$ In particular, the concordance of H could require a rescaling by a function of k
- Develop residuals analysis to choose between various distances.
- Theoretical analysis of NMF estimator in a statistical context : convergence, consistence...