Hi-C Differential Analysis: A new method using tree representation based on Contiguity Constrained Hierarchical Agglomerative Clustering (CCHAC)

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Pratical case and Data

Introduction

Starting point :

→ work and data of M. Marti-Marimon PhD thesis:

Study of fetal development of piglets using Hi-C data:

→ Data produced by Centre INRA - Occitanie Toulouse :

- 3 Hi-C samples corresponding to 90 days of gestation
- 3 Hi-C samples corresponding to 110 days of gestation

Aim of the hierarchical differential analysis method:

- overcome limits linked to methods based on bin pair level comparisons

State of the art

Introduction and notation

Main question of Hi-C differential analysis:

Given two sets of Hi-C matrices, corresponding respectively to two biological conditions, how can we compare those two biological conditions with statistical guarantees ?

Notation:

- Considered biological conditions: $\mathcal{C}_i$ for $i \in \{1, 2\}$
- Hi-C matrices: H^t for $t \in \{1, \ldots, T\}$
- Interaction Counts: $H^t = (h^t_{ij})_{1 \leq i,j \leq p}$ where p is the number of bins

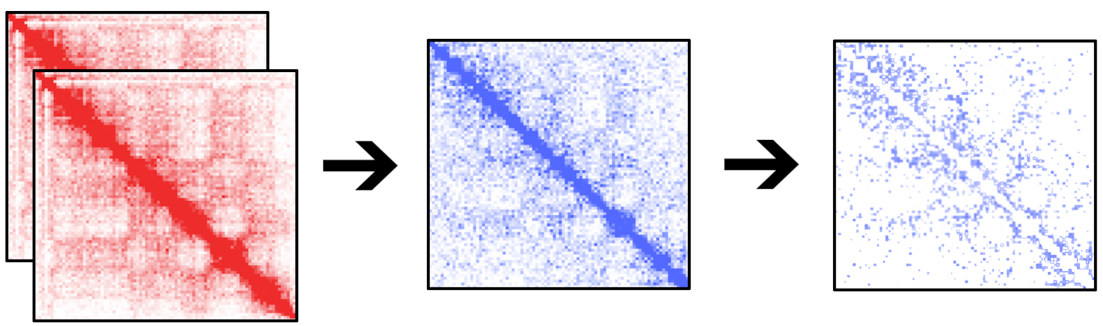
We have

- $\mathcal{C}_1 \cup \mathcal{C}_2 = \{1, \ldots, T\}$
- $\mathcal{C}_1 \cap \mathcal{C}_2 = \emptyset$

Bin pair level comparisons

Most methods realize comparisons at a **bin pair level**:

1. For each bin pair, compute a certain statistic
2. For each bin pair, deduce from the statistic a *p*-value
3. Apply correction for multiple testing
4. Obtain a list of differential bin pairs between the two conditions



Using Z scores

[Stansfield et al., 2018] developed a method implemented in the R package HiCcompare :
$\rightarrow$ cannot use replicate ($\mathcal{C}_1 = \{1\}$ and $\mathcal{C}_2 = \{2\}$)

1. For each bin pair (i, j), compute $m_{ij} = \log_2\left(\frac{h_{ij}^{\mathbf{2}}}{h_{ij}^{\mathbf{1}}}\right) = \log_2\left(h_{ij}^2\right) - \log_2\left(h_{ij}^1\right)$
2. For each bin pair, compute the associated Z-score:

$$z_{ij} = \frac{m_{ij} - m}{\sigma}$$

where m is the mean of the m_{ij}'s and σ their standard deviation

$\rightarrow$ deduce p-**values**

Limits:

- statistical guarantees are very limited
- does not account for intra-condition variability (no replicates)

Using $\mathcal{NB}$ distribution

[Lun and Smyth, 2015] developed a method implemented in the R package diffHic :
$\rightarrow$ can use replicates (at least 2 replicates by conditions)

1. Hi-C entries are modeled using negative binomial distributions:

$$h_{ij}^{t} \sim \mathcal{NB}(\mu_{ij}, \phi_{ij})$$

2. Test is performed identically as for RNA-seq

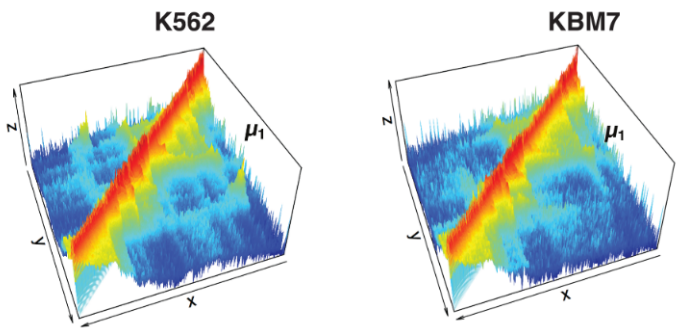
Limits:

- does not account for the depedency between bin pairs

Using the neighbouring structure of Hi-C maps

[Djekidel et al., 2018] developed a method implemented in the R package FIND :
$\rightarrow$ can use replicates (at least 2 replicates by conditions)

1. Represent counts h_{ij}^t by the triplet $(i, j, h_{ij}^t) \in \mathbb{R}^3$ and define $(i, j, \mu_{1/2})$ where $\mu_{1/2}$ is the mean of counts for the first/second condition



2. Statistical test based on a homogeneous spatial Poisson process
$\rightarrow$ similar to what is done in neuro-imaging comparisons.

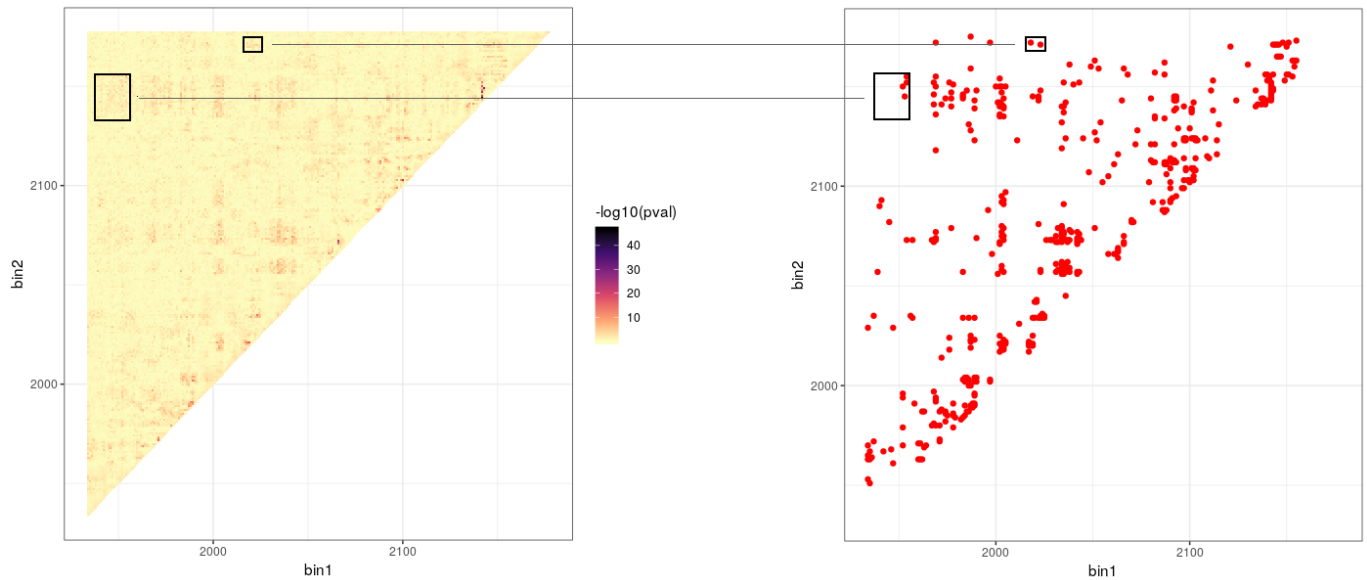
Limits:

- works well only if bin resolution is very high
- unsure that the model is well-suited for Hi-C data

Limits of comparisons at bin pair level

Results:

List of bin pairs (i, j) corresponding to differential interactions between conditions



Limits: These approaches do not account for:

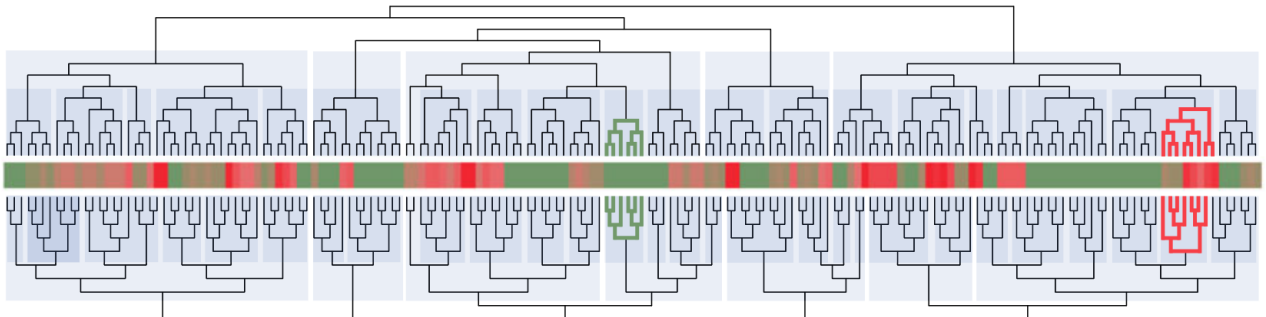
- Dependency between bin pairs
- Hierarchical structure of Hi-C data

$\Rightarrow$ Lack of interpretability in terms of structural differences

[Fraser et al., 2015]'s alternative

[Fraser et al., 2015] developed an approach based on tree structures which account for structural differences:
$\rightarrow$ cannot use replicate ($\mathcal{C}_1 = \{1\}$ and $\mathcal{C}_2 = \{2\}$)

1. For each Hi-C matrix, H^1 and H^2, obtain a clustering of the genome (e.g. TAD clustering)
2. Find common clusters between the two obtained clusterings
3. Apply a hierarchical clustering on those common clusters using the mean of interaction counts as a similarity measure:
   $\rightarrow$ Result : Tree of common clusters spatial organization for each sample
4. A score based on the comparison of path distances within the trees is associated to each cluster (Local Tree Changes measure) and Z-score are computed



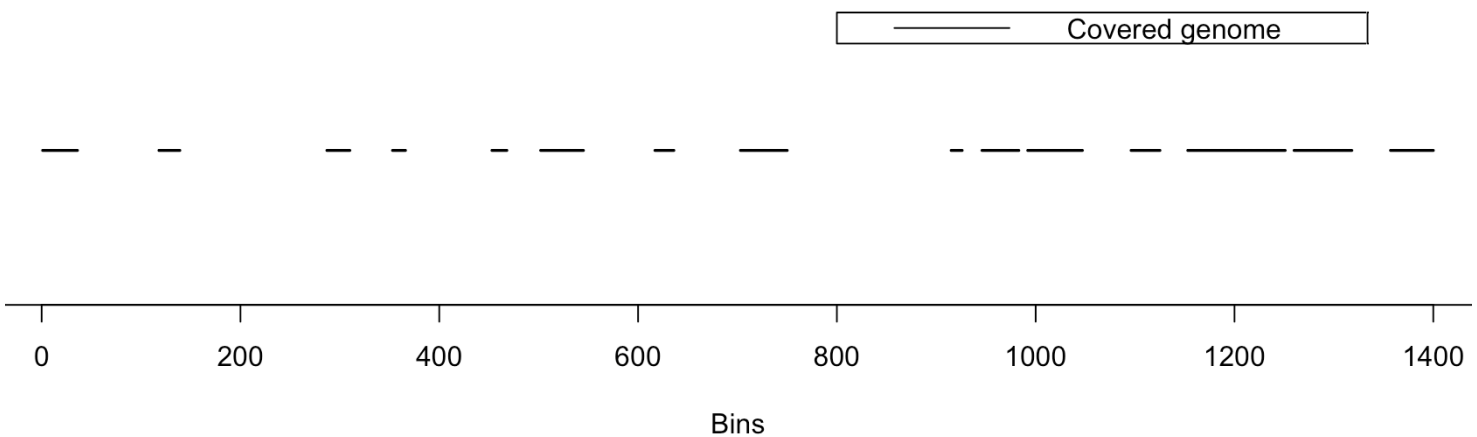
Limits of [Fraser et al., 2015]'s alternative

Results:

List of clusters of bins with differential reciprocal structural organization between conditions

Limits:

- does not account for intra-condition variability (no replicates)
- common structures typically represent a narrow part of the genome:
  $\rightarrow$ Differences probably also lie in regions that are rejected by this approach



Overcoming some of those limits ?

In order to overcome some previously listed limits, a method should be able to:

- perform **structural comparisons**
- use replicates in order to take into account **intra-condition variability**

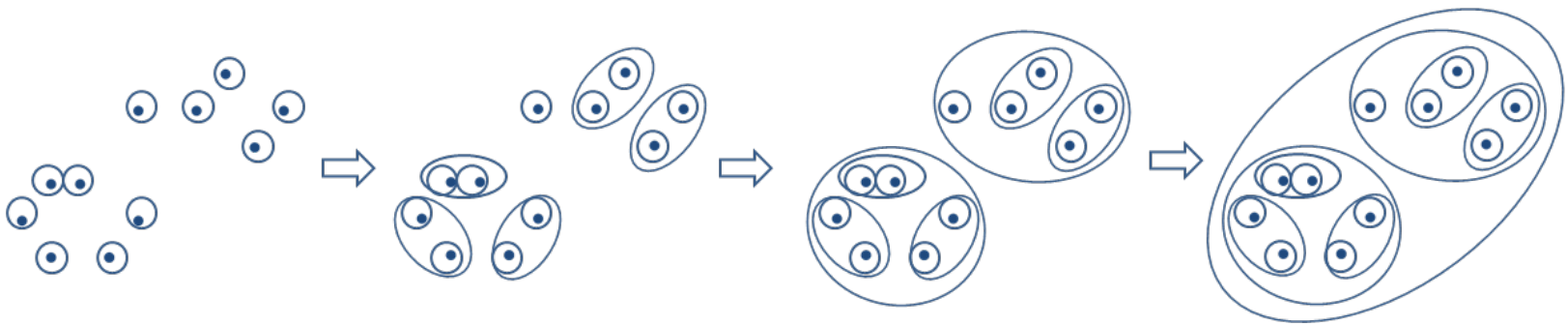
$\rightarrow$ The method proposed in the sequel is also based the **comparisons of tree structures** and can use **replicates**

Differential Analysis method based on CCHAC

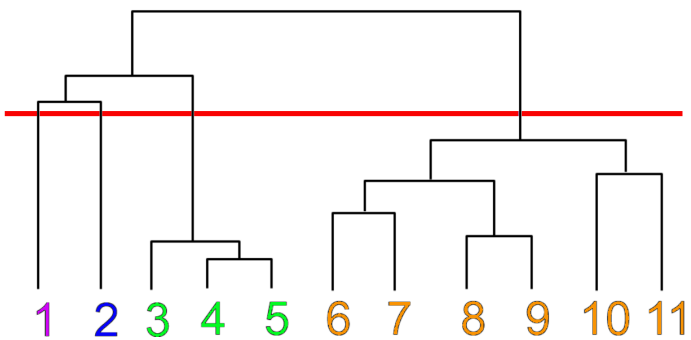
Hierarchical Agglomerative Clustering (HAC)

A multiscale approach to study hierarchical structure:

Initialisation: **For** $t = 1, \ldots, n$ **:** **End:**



Graphical representation of HAC results: $\rightarrow$ **Dendrograms**



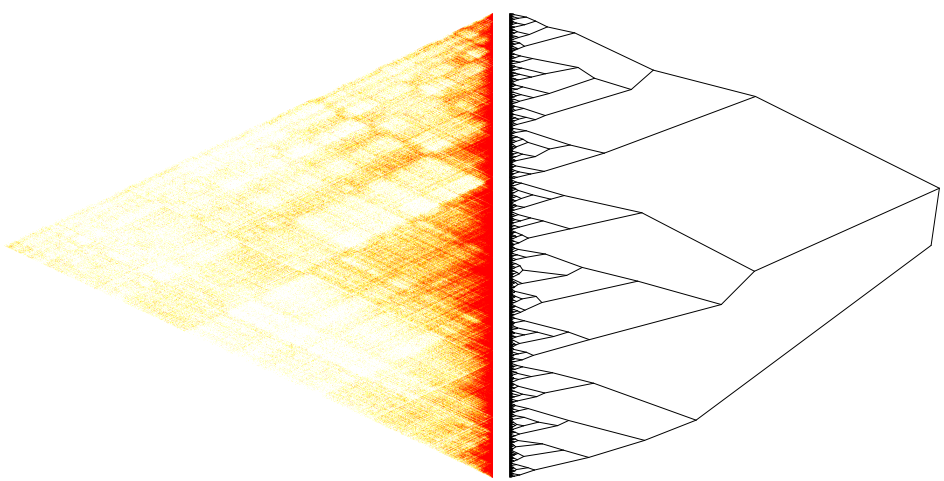
Hi-C and CCHAC

Hi-C data are **3D-proximity measure** $\leftrightarrow$ **similarity** data
$\Rightarrow$ Statistically founded possibility to use HAC on Hi-C matrices
[Randriamihamison et al., 2019]

Contiguity **C**onstrained **H**ierarchical **A**gglomerative **C**lustering:
$\rightarrow$ only adjacent bins can be merged

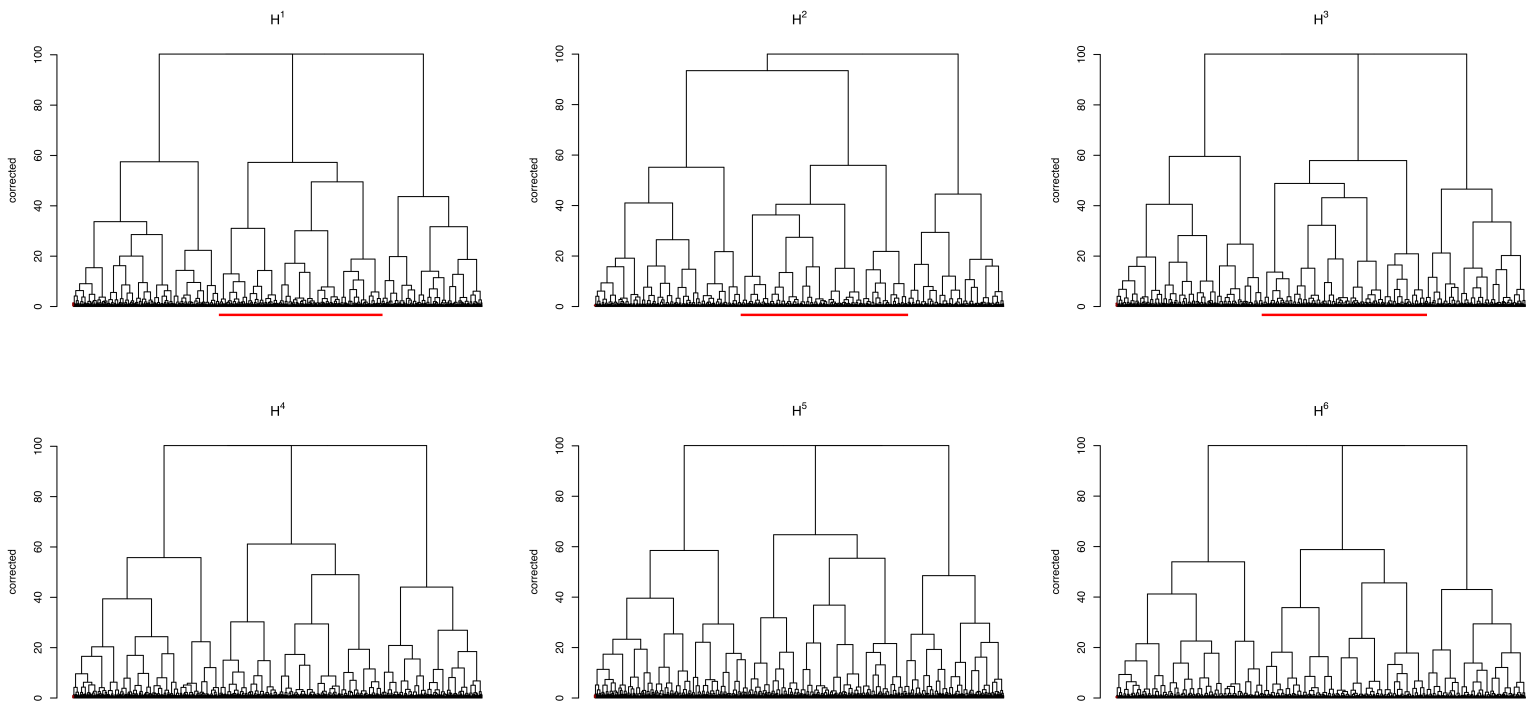
Implementation: R package adjclust

Using CCHAC on Hi-C matrices produces binary trees:



Overview of the method

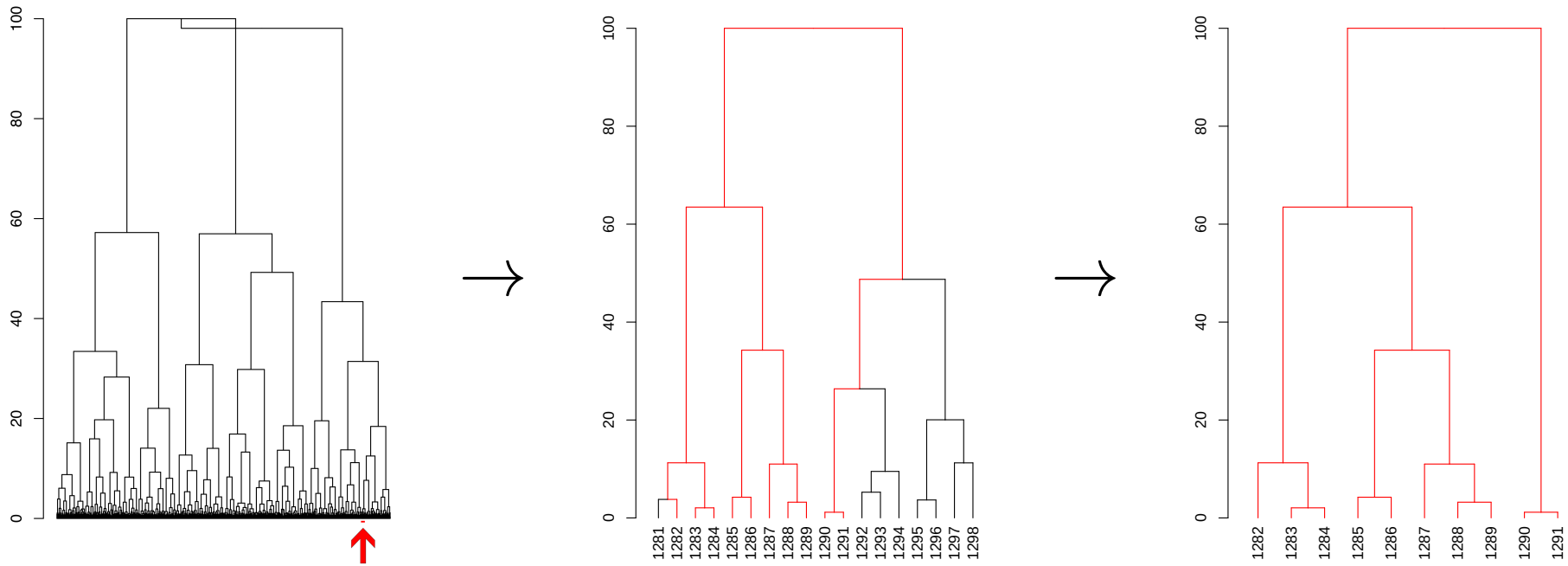
1. For each Hi-C Matrix, obtain a dendrogram using CCHAC
2. For each dendrogram and for each genomic region under study (e.g. all genomic intervals of a fixed bin size), consider the associated induced subtrees
3. Using distances between induced subtrees, compute a statistic to compare biological conditions on the genomic region



Defining induced subtrees

Given a dendrogram and a genomic interval, we can define an **induced subtree**:

→ Example for genomic interval $[1282, 1291]$:



→ Result: a set of 6 induced subtrees (one for each sample) defined on the same genomic interval

Comparing induced subtrees

Comparison of 6 corresponding induced subtrees (defined on the same genomic interval) $\Rightarrow$ Need for a **tree distance**

A lot of possible tree distances:

- R package ape
- R package distory

Simulation $\rightarrow$ **Weighted Path Difference Metric (WPD)**

Practical case (2×3 samples):
For each genomic interval, we obtain:

- 6 intra-conditions distances
- 9 inter-conditions distances

Defining a statistic [work in progress]

A solution might be to consider a statistic such as:

$$W_l := \frac{\bar{d}_l^{inter} - \bar{d}_l^{intra}}{\sigma_{d_l}}$$

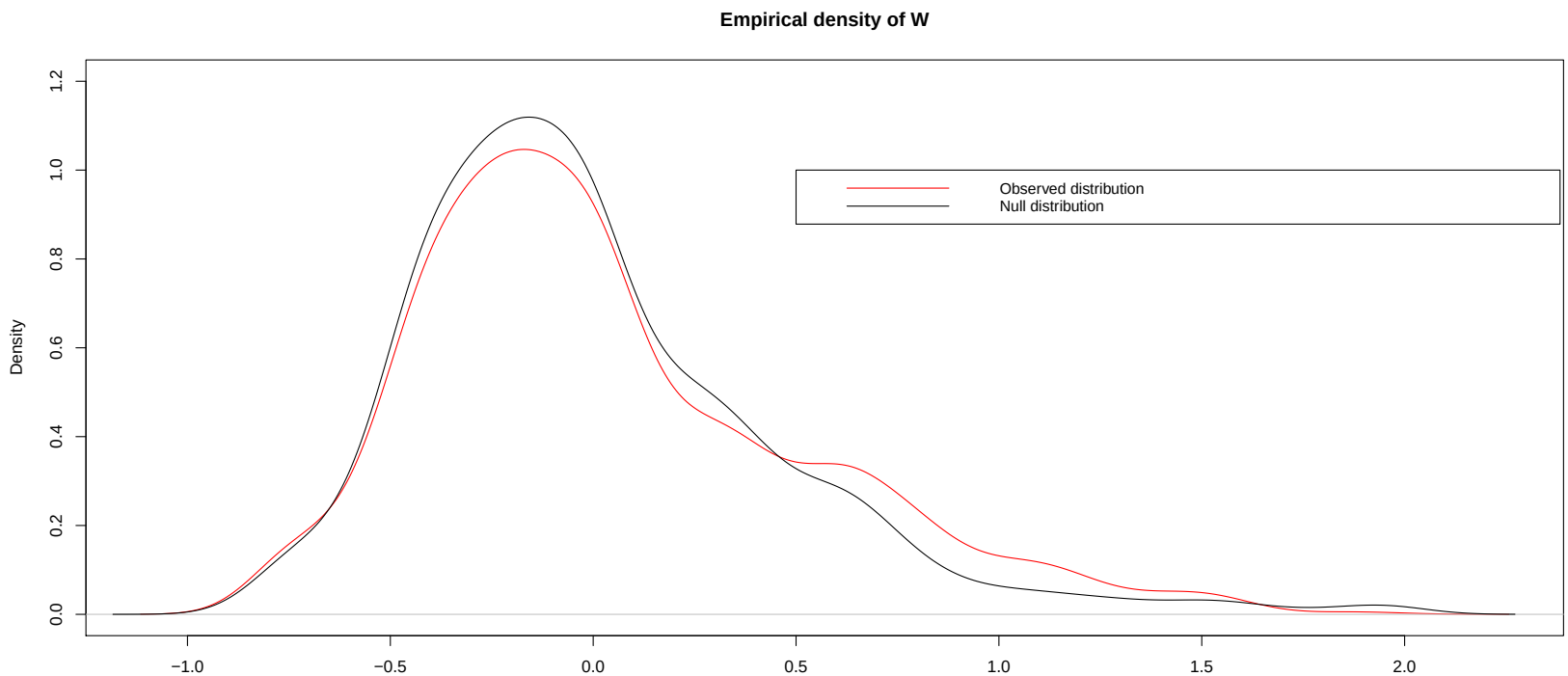
where

- $\bar{d}_l^{inter}$ is the mean of d_l entries corresponding to inter-conditions distances
- $\bar{d}_l^{intra}$ is the mean of d_l entries corresponding to intra-conditions distances
- σ_{d_l} is the standard deviation of d_l entries

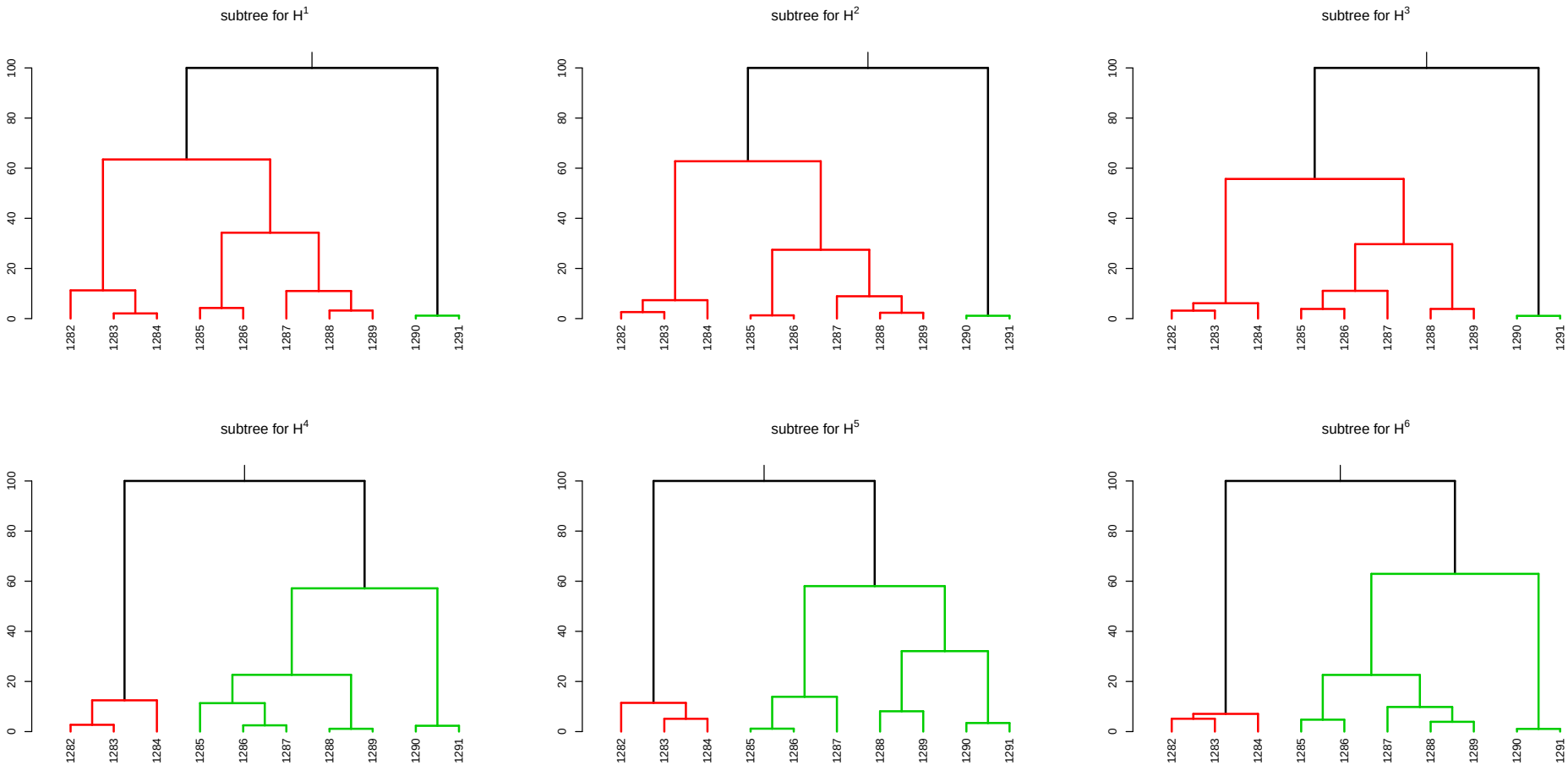
Empirical distribution of W

Setting:

- data from fetal pig development ($\mathcal{C}^1 = \{1, 2, 3\}$, $\mathcal{C}^2 = \{4, 5, 6\}$)
- bin resolution: 40 kb
- chromosome 18
- genomic intervals defined by sizes: 10 bins, 20 bins



Example of a "differential structure"



Conclusion

- What we wanted: a method that would allow to:
  - structurally interpret differences
  - use replicates

- The answer: Differential Analysis based on CCHAC [work in progress]:
  - based on tree representation of Hi-C data obtained via CCHAC
  - focus on genomic intervals in order to allow local comparisons
  - select genomic intervals over which the 3D-structure of genome is differential

Further investigations:

- How to choose a relevant set of genomic intervals for the analysis ?
- Alternative choice of the test statistic (percentage of explained inertia ?)
- Extension of the study to whole genome

Thank you for your attention!

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Empirical density of W for biological conditions defined as different cell lines: