Comparative analysis of 3D genomic data

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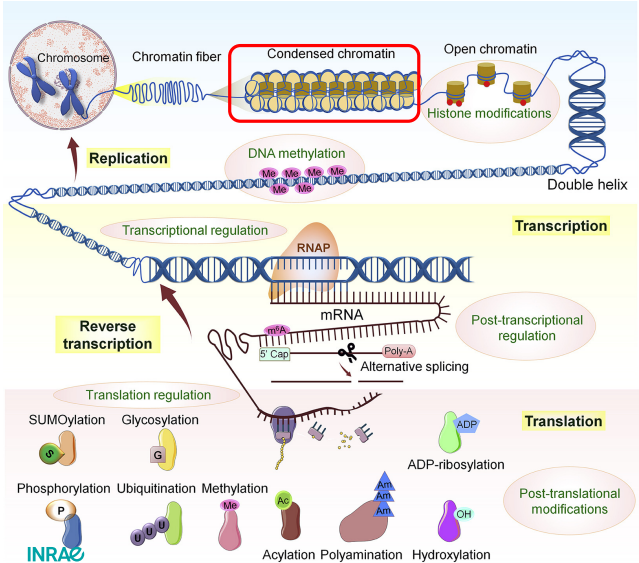
Liberté Égalité Fraternité

INRAE

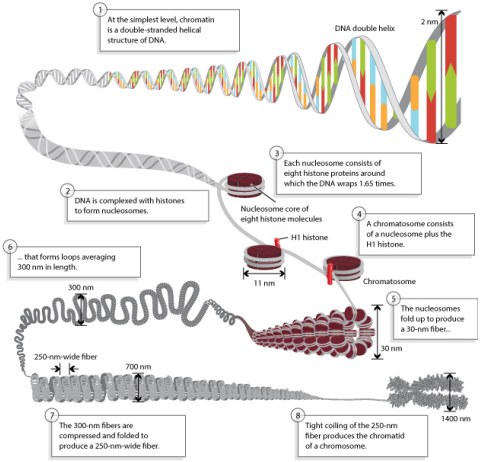
Outline

3D organization of DNA as a regulatory layer



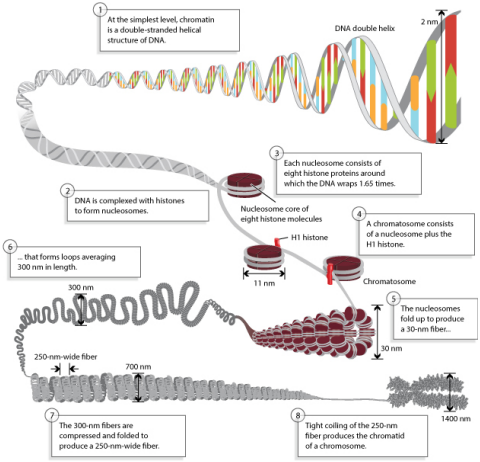
[Pramanik et al., 2021]

DNA organization



[Annunziato, 2008] 2 meters compressed in 6 μm

DNA organization



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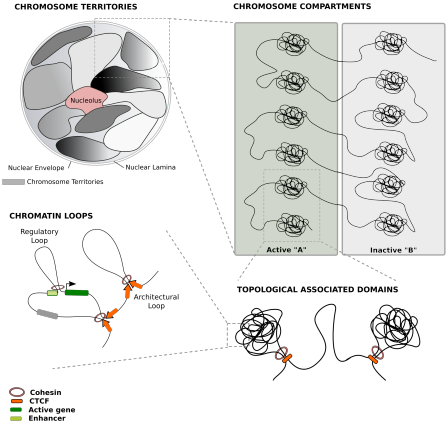
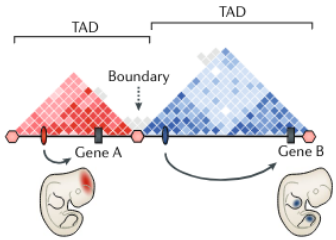
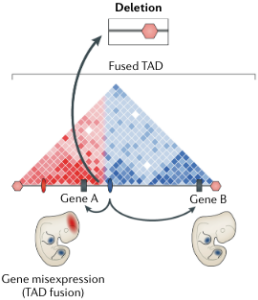
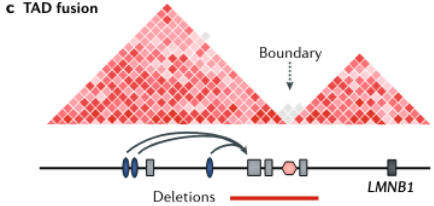


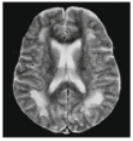
[**Servant, 2017**] adapted from
[**Bonev and Cavalli, 2016**]

Impact on DNA functionning [Spielmann et al., 2018]



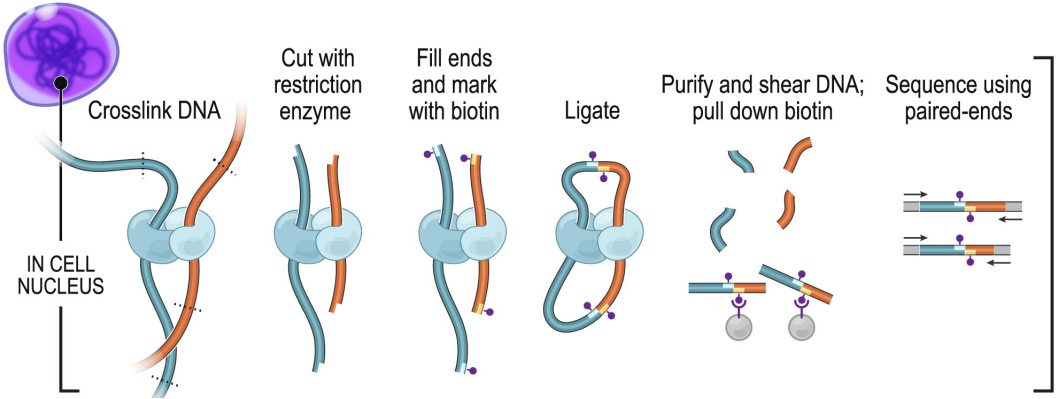




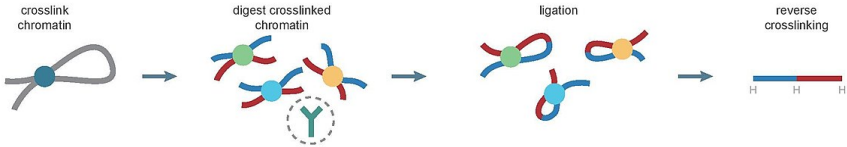


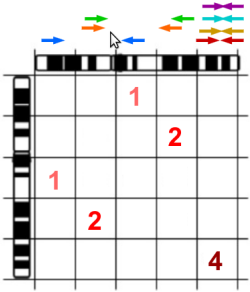
Adult-onset demyelinating leukodystrophy

Hi-C experiment



Hi-C data





Images: adapted from **[Li et al., 2014]** (top) and courtesy of Sylvain Foissac + own work (bottom).

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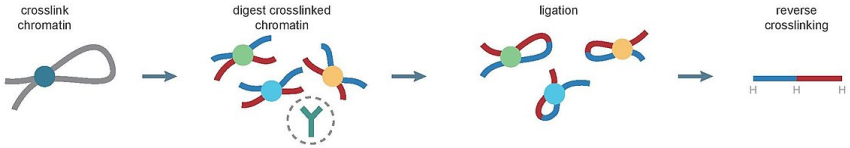

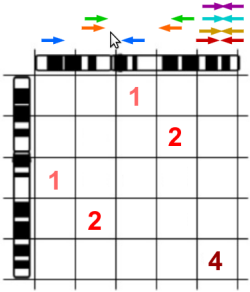

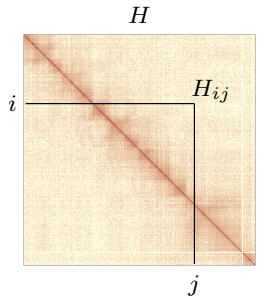

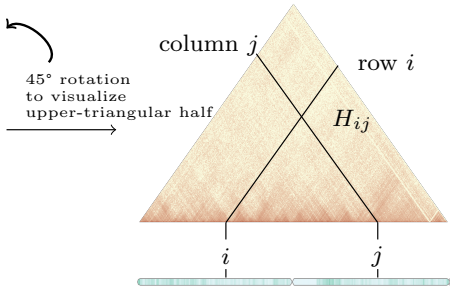

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Outline

Where Statistics comes into play

Data: $(H_{ij})_{ij}$, symmetric matrix where entries are counts

H_{ij}: proxy of the spatial proximity between genomic position i and j (call "bins"; (i, j) is a "bin pair" or a "pixel")

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Series of works on:

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Hi-C data clustering







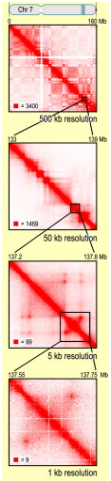
INRAE Inria cnrs

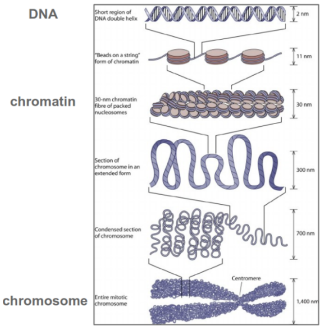
Statistical tests for Hi-C data

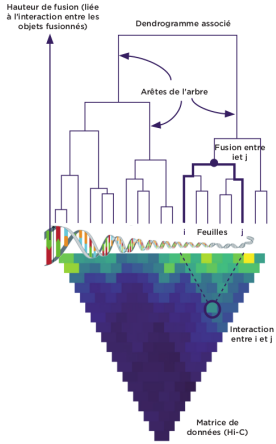



INRAE

1. Hi-C matrices and hierarchical clustering [Neuvial et al., 2023]







Idea used in: [**Fraser et al., 2015**, **Soler-Vila et al., 2020**, **Zhang et al., 2021b**] among others

Clustering based on Hi-C matrices

Summary of the work performed in: Projet CNRS SCALES (with P. Neuvial & S. Foissac). Thèse INRAE/Inria Nathanaël Randriamihamison (also with M. Chavent)

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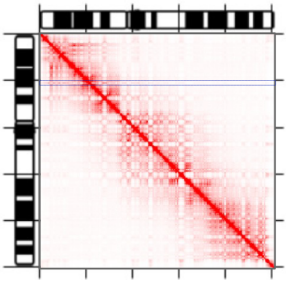
How is hierarchical clustering usually performed on Hi-C matrices?



1. a bin =
2. Euclidean distance + HC

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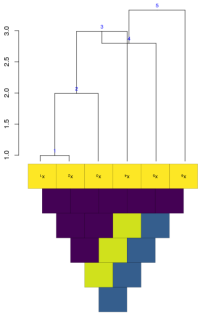
Why is it not satisfactory?

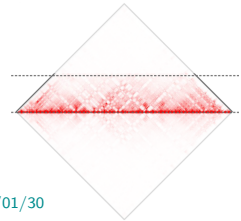
1. Hi-C matrices are already distances!
2. sparse vs full representations

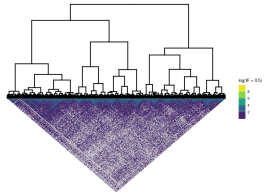
Clustering based on Hi-C matrices

Contributions

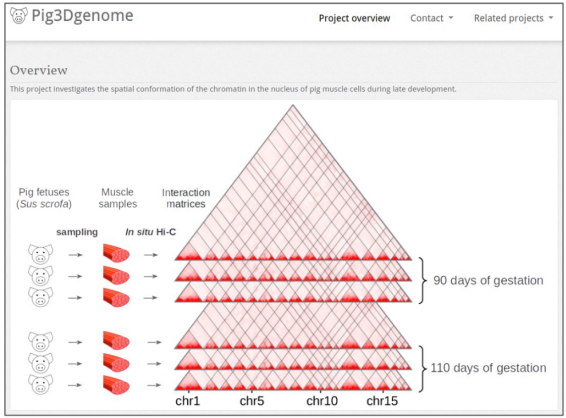


1. extend Ward's HC to similarity matrices as Hi-C with adjacency constraint **[Randriamihamison et al., 2021]**
2. study of the mathematical properties **[Randriamihamison et al., 2021]**
3. study of reversals in practice on Hi-C matrices **[Randriamihamison et al., 2021]**
4. fast version using a band computation **[Ambroise et al., 2019]**, implemented in the R package **adjclust**





2. Motivation example for tests in Hi-C data: Pig3D genome project



Marti-Marimon et al, 2021

Pig3Dgenome

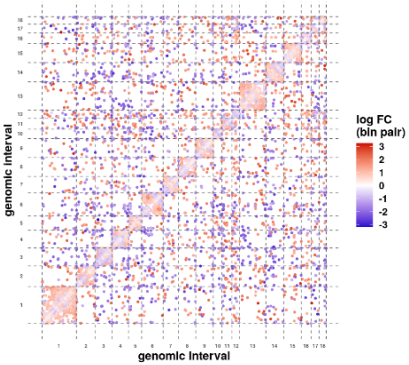
Differences between conditions?

[Marti-Marimon et al., 2018] (courtesy of Sylvain Foissac)

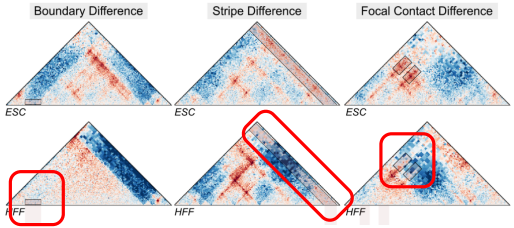
Outline

Standard methods for differential analysis of Hi-C data

Pixel-based methods



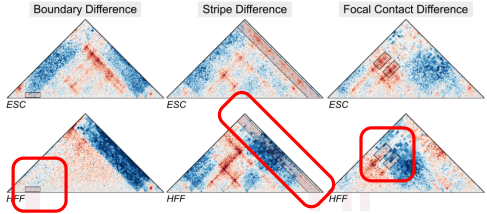
Structure-based methods



[Gjoni et al., 2025]

Structure-based methods

- target a specific structure



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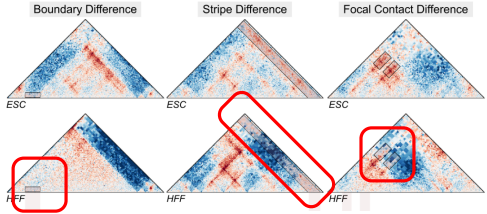


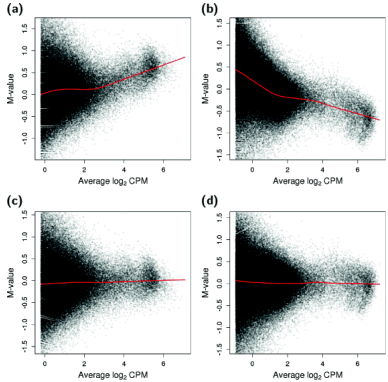
- limited to this specific structure and sensitive to differences in methods for structure-discovery **[Forcato et al., 2017]**

Pixel-based methods [Jorge et al., 2025]

	Design		Preprocessing		Model				p-values	
Tool	Accept $K > 2$ conditions	Usage of covariates	Filtering	Normalization	Assumption on data distribution	Usage of replicates	Structure-aware	Type of method	raw p-values provided	p-value correction
ACCOST	✘	✘	bad quality	ICE	NB	✔	✘	exact test	✔	✘
CHESS	✘	✘	bad quality	ICE	✘	✘	✔	Z-score	✔	✘
diffHic	✔	✔	low counts	MA	NB	✔	✘	GLM	✔	BH
FIND	✘	✘	✘	KR	spatial Poisson process	✔	✔	exact test	✘	BH/distance
HiCcompare	✘	✘	user-defined	MD	✘	✘	✘	Z-score	✔	BH/distance
HiCDCPlus	✘	✘	bad quality	ICE	NB	✔	✘	exact test	✔	BH
HOMER	✘	✘	✘	ICE	✘	✘	✘	Z-score	?	?
multiHiCcompare	✔	✔	low counts	MD	NB	✔	✘	GLM	✔	BH/distance
Selfish	✘	✘	low counts	KR	✘	✘	✔	Z-score	✘	BH
sslHiC	✘	✘	large distance	min/max	✘	✘	✔	GNN	✘	BH

Most accurate method: **diffHic** [Lun and Smyth, 2015]

1. normalize data with MA trend correction by cyclic LOESS



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2. fit a BN GLM: $H_{i,j}^{\ell} \sim \mathrm{BN}(\lambda_k, \phi_{i,j,k})$, $k := \mathrm{Condition}(\ell)$, $\phi_{i,j,k}$: dispersion parameter ($+$ moderated tests)

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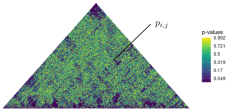
$\Rightarrow \mathbf{P} = (p_{ij})_{i,j=1,\ldots,p,\, i \leq j}$



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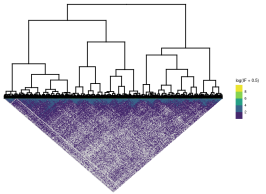
Second approach **[Neuvial et al., 2024]**



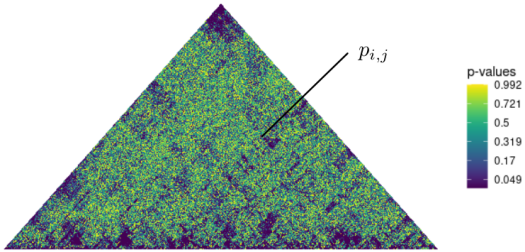
But:

- only partially exploits the spatial structure
- limited to TAD-like structures

Outline

General objective

What do we start from?



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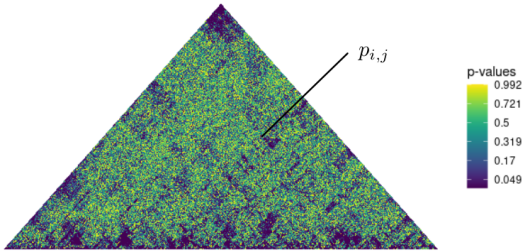
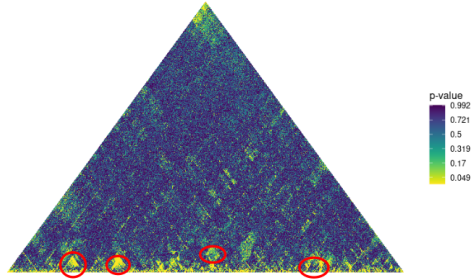


What do we want to achieve?

1. provide statistical guarantees for any (arbitrary) cluster



2. build clusters in a data-driven manner

Why post-hoc inference?

$\mathcal{H} = \{(i,j) : \ i,j = 1,\dots,p, \ i \leq j\}$: hypotheses to test
$\mathcal{H}_0 \subset \mathcal{H}$: true null hypotheses

Multiple testing correction:

- $\mathcal{R}$: rejected hypotheses
- False positives: $\mathcal{R} \cap \mathcal{H}_0$
- False Discovery Rate: $\mathbb{E}\big(\frac{|\mathcal{R} \cap \mathcal{H}_0|}{|\mathcal{R}| \vee 1}\big)$.

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Post-hoc inference

[Goeman and Solari, 2011]:

- For $S \subset \mathcal{H}$, quantify $\mathrm{TDP}(S) = 1 - \frac{|S \cap \mathcal{H}_0|}{|S|}$?
- post-hoc bound $V_\alpha : S \subset \mathcal{H} \to \mathbb{R}$ st:

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$$\mathbb{P}(\forall S \subset \mathcal{H}, |S \cap \mathcal{H}_0| \leq V_\alpha(S)) \geq 1 - \alpha$$

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$$\mathbb{P}(\forall S \subset \mathcal{H}, \mathrm{TDP}(S) \geq \gamma_\alpha(S)) \geq 1 - \alpha$$

with $\gamma_\alpha(S) = 1 - \frac{V_\alpha(S)}{|S|}$.

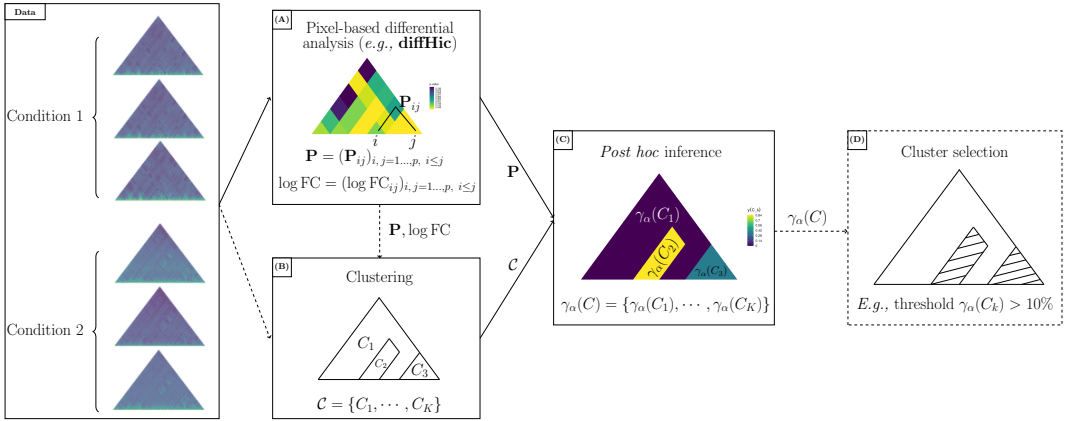
A very general post-hoc bound

Under independence or PRDS **[Benjamini and Yekutieli, 2001]**, Simes' post hoc bound:

$$V_\alpha^{\text{Simes}}(S) = \min_{1 \le \ell \le |S|} \left[\sum_{(i,j) \in S} \mathbb{1}_{\{\mathbf{P}_{ij} > \frac{\alpha \ell}{m}\}} + \ell - 1 \right]$$

is valid **[Goeman and Solari, 2011]**.

In short, proposed procedure



Data driven clustering

Desirable properties of clusters:

- contiguous 2D regions in Hi-C map
- related to bin pair test results
- also accounts for direction of the change (p-value not sufficient)

Data driven clustering

2D constrained hierarchical clustering

[Lebart, 1978, Grimm, 1987, Gordon, 1996, Thirion et al., 2014]

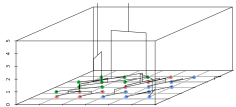
1. **Neighboring constraint:**

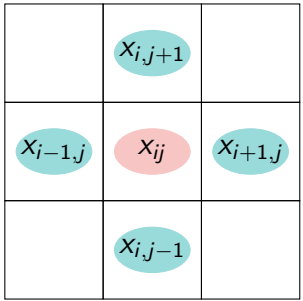
$$(i,j) \text{ and } (i',j') \text{ neighbors } \iff |i-i'|+|j-j'|=1$$

2. **Agglomerative clustering** based on
   - **counts**:

$$x_{ij} := (H_{ij}^{C_1,r_1}, H_{ij}^{C_1,r_2}, H_{ij}^{C_1,r_3}, H_{ij}^{C_2,r_1}, H_{ij}^{C_2,r_2}, H_{ij}^{C_2,r_3})$$

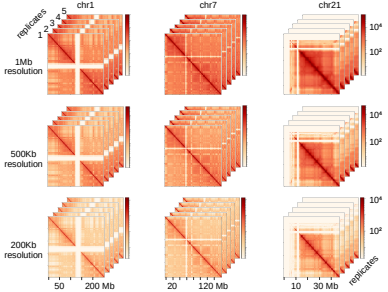
   - **diff**: $x_{ij} = (\log \mathrm{FC}_{ij}, \mathbf{P}_{ij})$

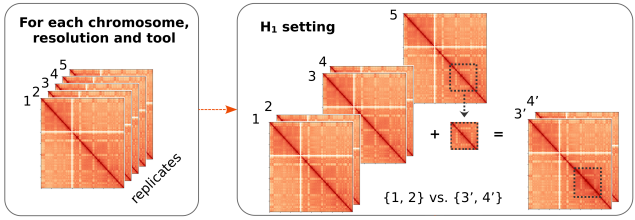




Simulation study

Semi-simulated data from ENCODE data:





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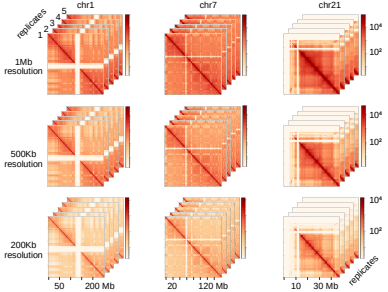
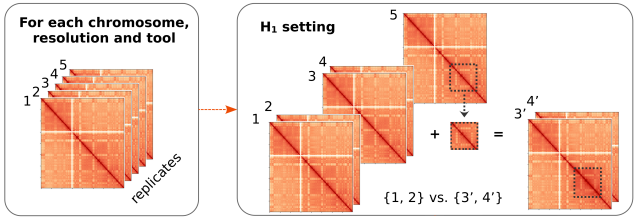
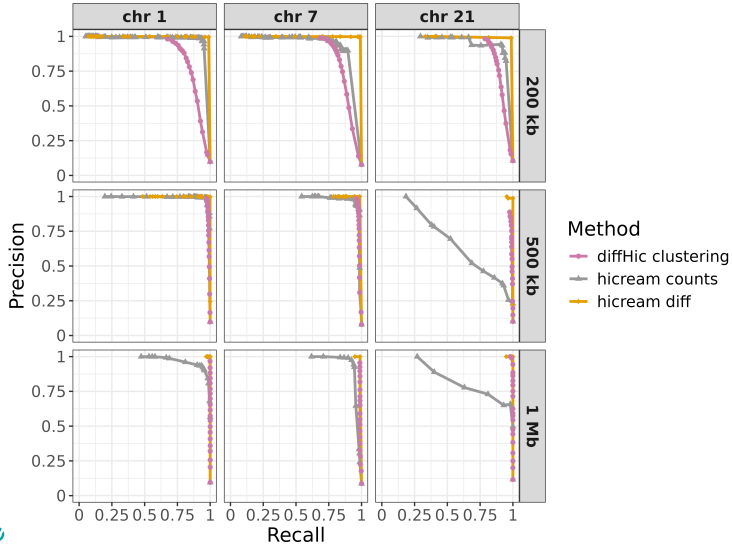




Tested methods:

- our : constrained 2D clustering + $\gamma_\alpha(S)$ cutoff
- **diffHic** clustering based on adjusted p-values + cluster-level FDR cutoff

Results: PR curve



Results: numerical performance of "best" results

		diffHic clustering					hicream diff				
Chr.	Res.	Cluster-level FDR	Nb. of clusters	Median cluster size	Precision	Recall	TDP	Nb. of clusters	Median cluster size	Precision	Recall
1	200 kb	0.40	14198	3	0.879	0.802	0.014	120	36	0.996	0.989
1	500 kb	0.05	3037	4	0.988	0.971	0.086	72	70	0.998	0.999
1	1 Mb	0.05	2634	1	0.967	0.999	0.2	76	10.5	1	1
7	200 kb	0.45	3994	4	0.904	0.802	0.005	76	33	0.997	0.991
7	500 kb	0.05	1131	4	0.987	0.962	0.258	153	10	1	0.986
7	1 Mb	0.05	1109	1	0.955	0.987	0.698	42	11	1	0.988
21	200 kb	0.25	314	5	0.939	0.833	0.291	21	34	0.989	0.988
21	500 kb	0.05	151	2	0.888	0.975	0.083	19	10	0.988	1
21	1 Mb	0.10	100	1	0.99	0.99	0.2	7	12	1	1

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Real dataset with external biological knowledge

Data from: **[Zhang et al., 2021a]** (CTCF depletion study)

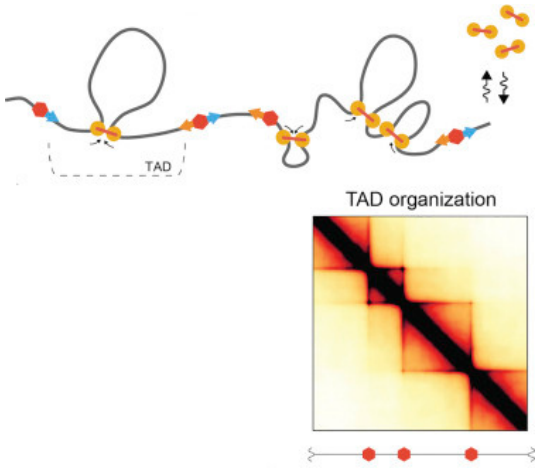
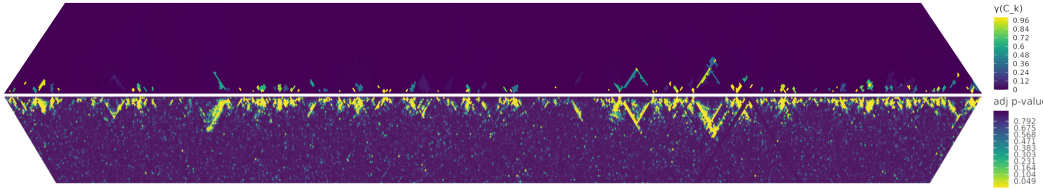


image adapted from: **[Fudenberg et al., 2016]**

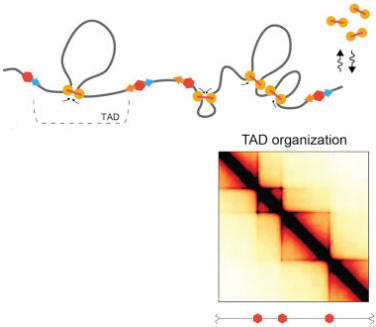
Smoothing property of **hicream**

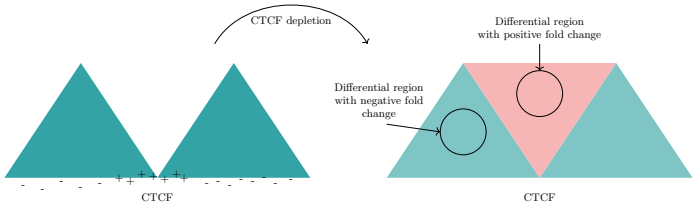
Minimal True Discovery Proportions from *post hoc* bounds



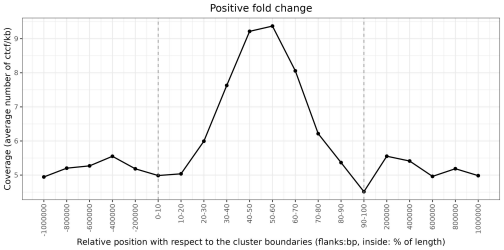
diffHic differential analysis adjusted p-values

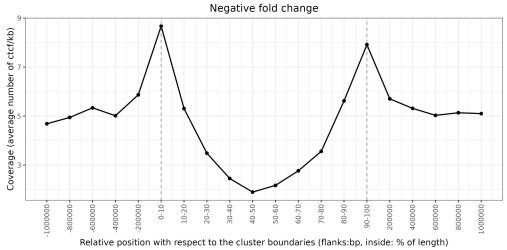
What to expect from CTCF depletion?

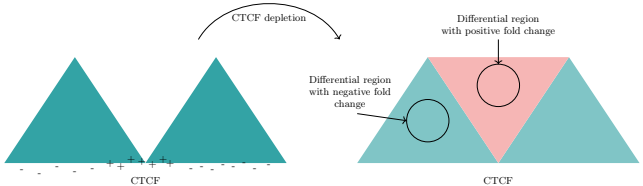




Results based on identified clusters







Conclusion and perspectives

Take home messages

- Hi-C data are challenging (hierarchical, spatial and counts)
- Post-hoc bound for genomics give interesting exploratory results with quantifiable statistical evidence (best of two worlds)

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Thank you for your attention!
Questions?

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