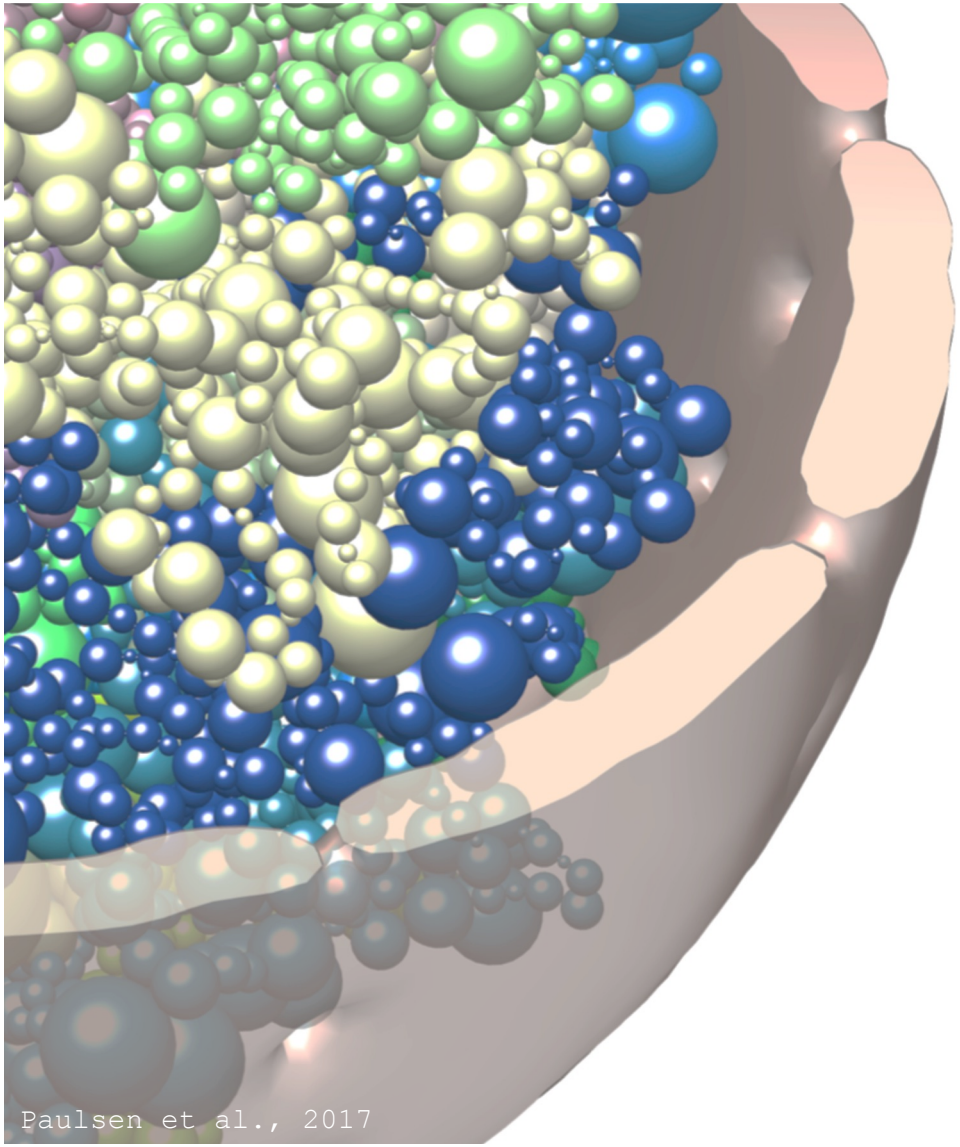


A primer on 3D genomics

UNIVERSITY OF COPENHAGEN

MARIE CURIE ACTIONS

✨ ***A mini-workshop***

DTU - NGS analysis course

9th January, 2026

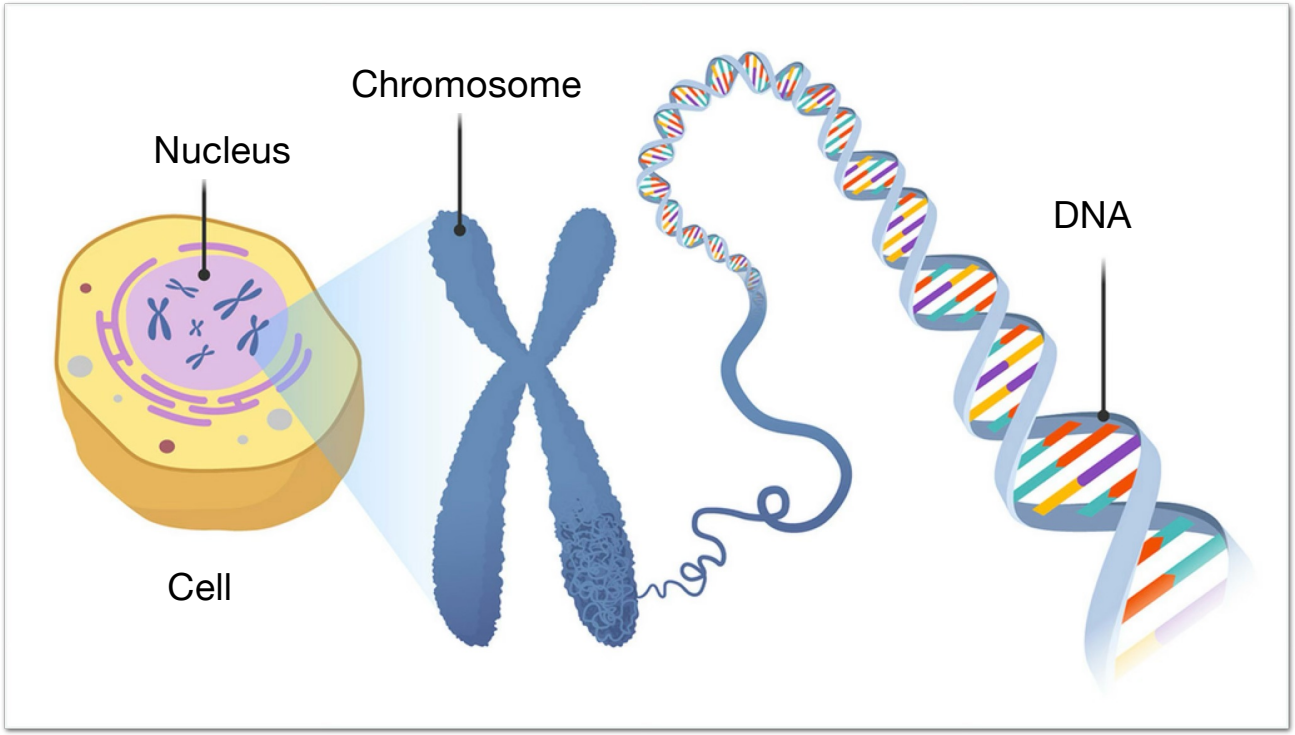
Juan Antonio Rodríguez

Asst. Professor at Globe Institute, KU

Why is structure important?

- Structure is more conserved than sequence
- Similar structures tend to have similar function.

DNA organizes in the nucleus



DNA organizes in the nucleus

How long do you think DNA inside a human cell is? **~2 m.**

Seiglerfone · 6y ago

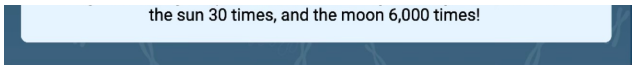
The distance to the moon is 363,104 km to 405,696 km from the Earth.

The length of a human's DNA is 2-3m.

There is one copy of DNA in a cell normally (not during replication) and there are 37.2T cells in the human body.

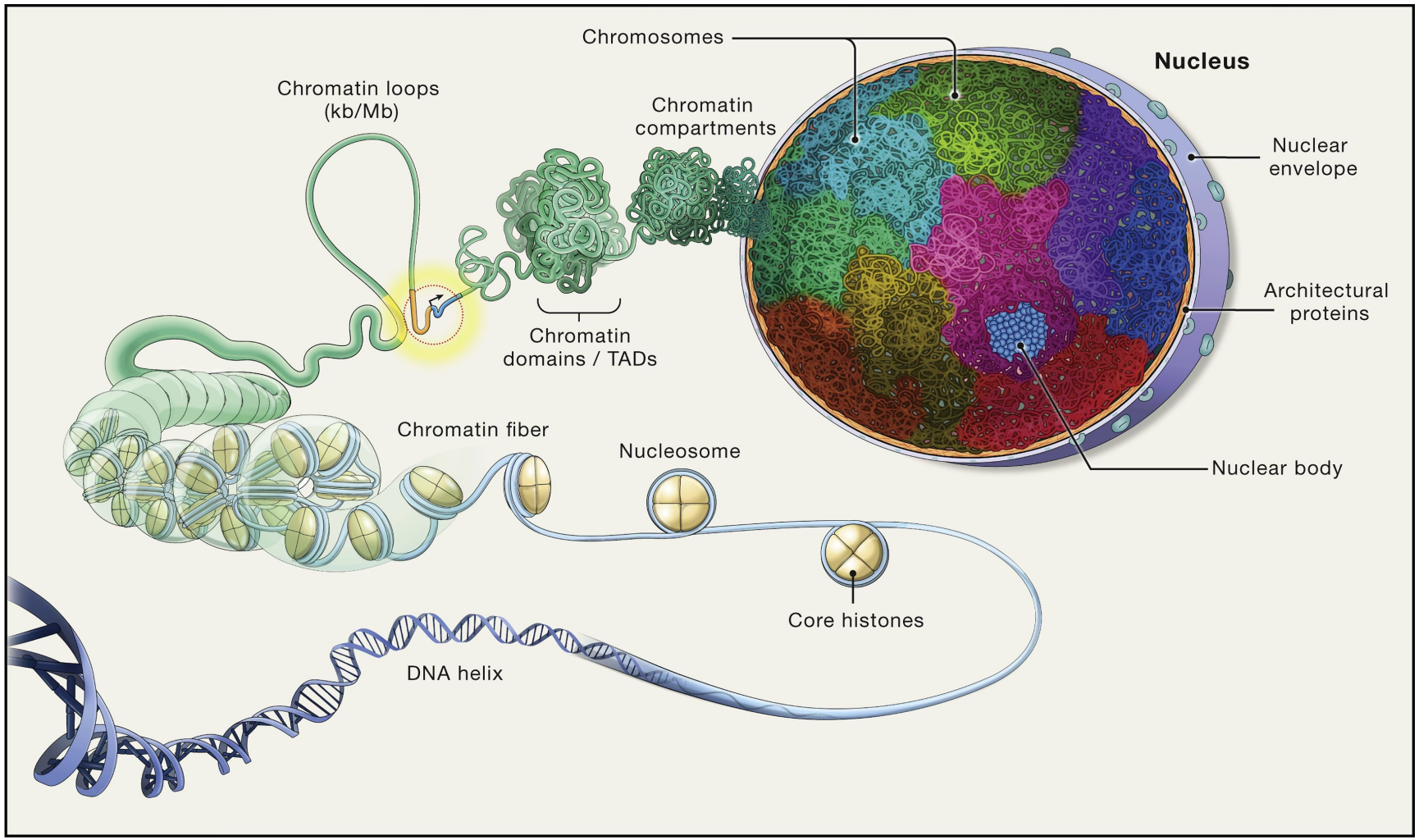
2 to 3 meters x 37.2T = 74,400,000,000 km to 111,600,000,000 km. Even if we only use the farthest away the moon is, and the lower estimate for DNA length, that's still over 91694 trips to the moon and back.

2 Reply Share ···



Why is the 3D genome structure important?

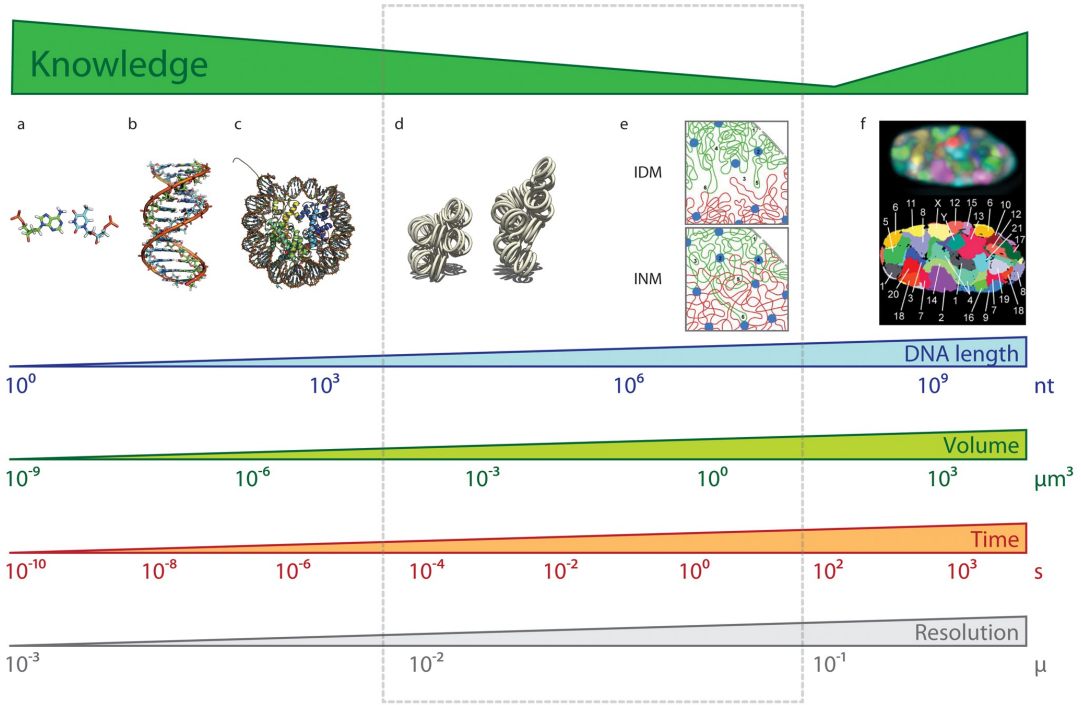
- ✓ Gives information on cell and metabolic regulation
- ✓ Tells us about the expressed genes on a tissue in a certain timepoint
- ✓ Helps us with assemblying new *de novo reference* genomes
- ✓ Fundamental to understand embryo development



Misteli, *Cell*, 2020. 10.1016/j.cell.2020.09.014

The resolution gap:

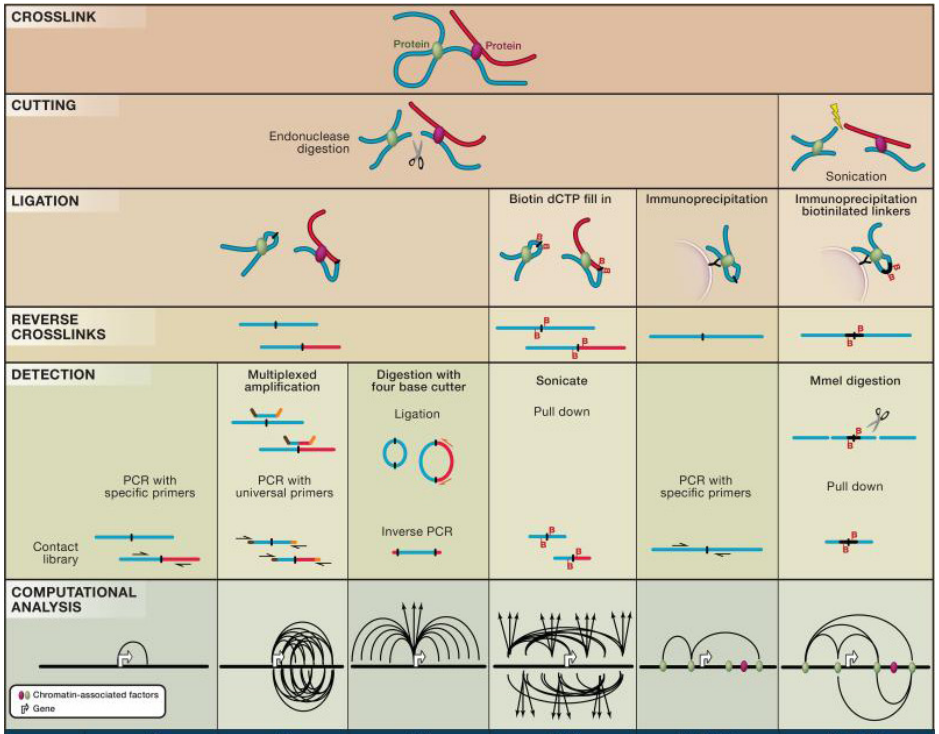
Techniques used to study 3D DNA conformation. Knowledge gap in the between?



Martí-Renom & Mirny, *PLOS Comp Bio*, 2011. 10.1371/journal.pcbi.1002125

Chromosome Conformation Capture Techniques

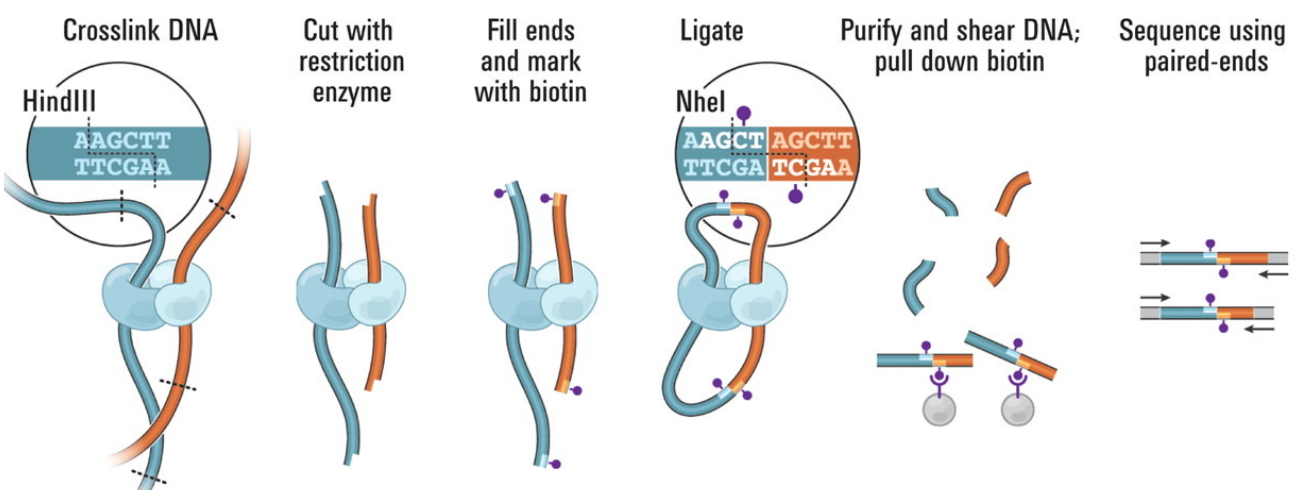
Or... 3C derived techniques.

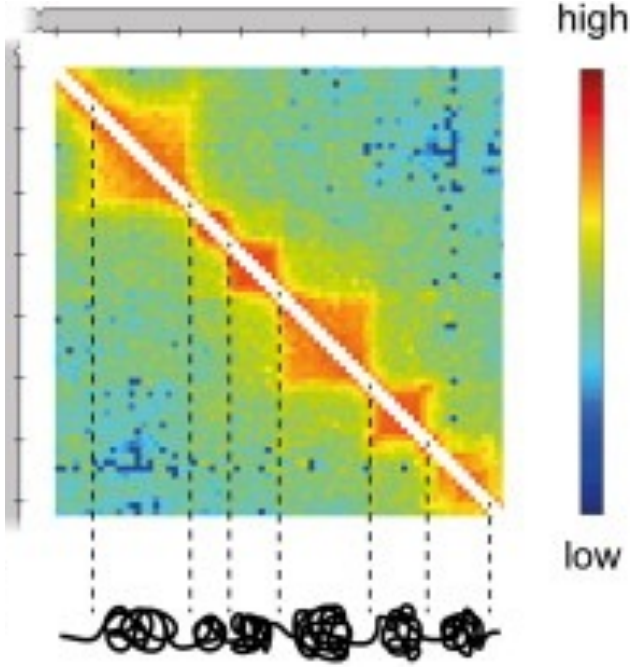


	3C	5C	4C	Hi-C	ChIP-loop	ChIA-PET
Principle	Contacts between two defined regions[3,17]	All against all[4,18]	All contacts with a point of interest[14]	All against all[10]	Contacts between two defined regions associated with a given protein[8]	All contacts associated with a given protein[6]
Coverage	Commonly < 1Mb	Commonly < 1Mb	Genome-wide	Genome-wide	Commonly < 1Mb	Genome-wide
Detection	Locus-specific PCR	HT-sequencing	HT-sequencing	HT-sequencing	Locus-specific qPCR	HT-sequencing
Limitations	Low throughput and coverage	Limited coverage	Limited to one viewpoint		Rely on one chromatin-associated factor, disregarding other contacts	
Examples	Determine interaction between a known promoter and enhancer	Determine comprehensively higher-order chromosome structure in a defined region	All genes and genomic elements associated with a known LCR	All intra- and interchromosomal associations	Determine the role of specific transcription factors in the interaction between a known promoter and enhancer	Map chromatin interaction network of a known transcription factor
Derivatives	PCR with TaqMan probes[7] or melting curve analysis[1]		Circular chromosome conformation capture[20], open-ended chromosome conformation capture[19], inverse 3C[12], associated chromosome trap (ACT)[11], affinity enrichment of bait-ligated junctions[2]	Yeast[3,15], tethered conformation capture[9]		ChIA-PET combined 3C-ChIP-cloning (6C);[16] enhanced 4C (e4C)[13]

Hakim & Misteli, *Cell,* 2012

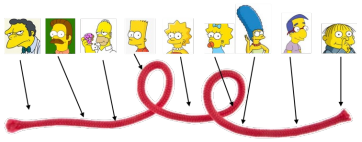
HiC-technique 101

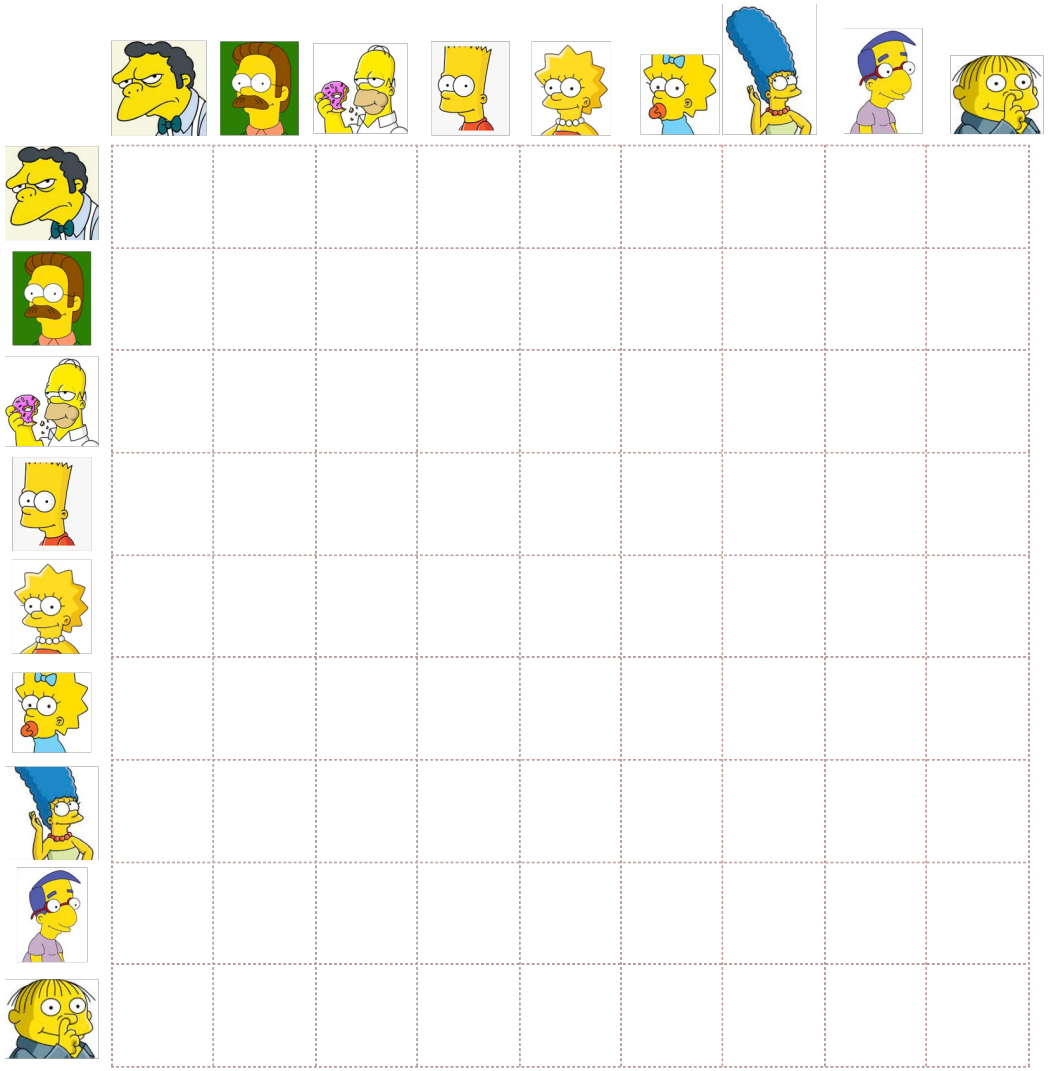


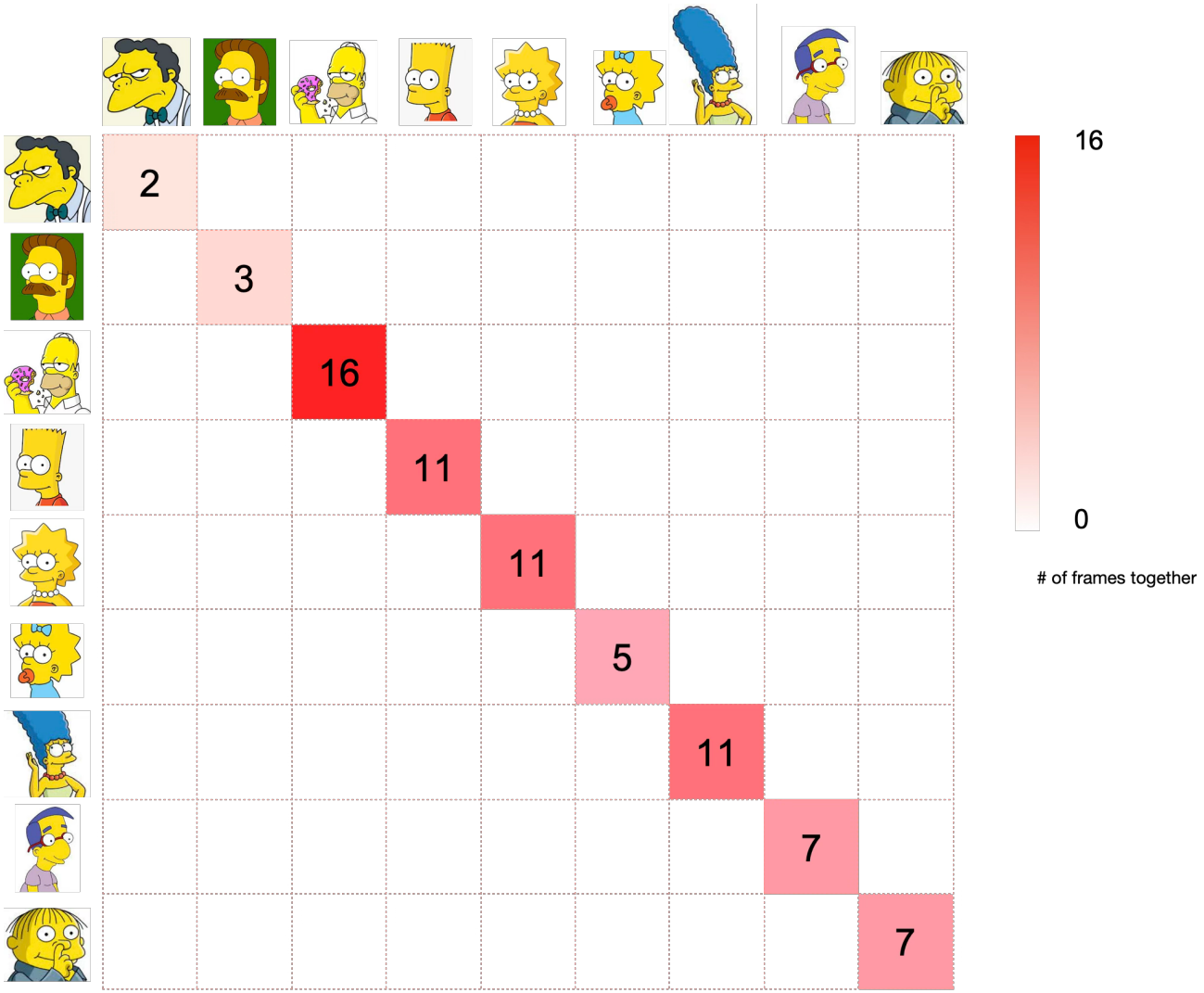


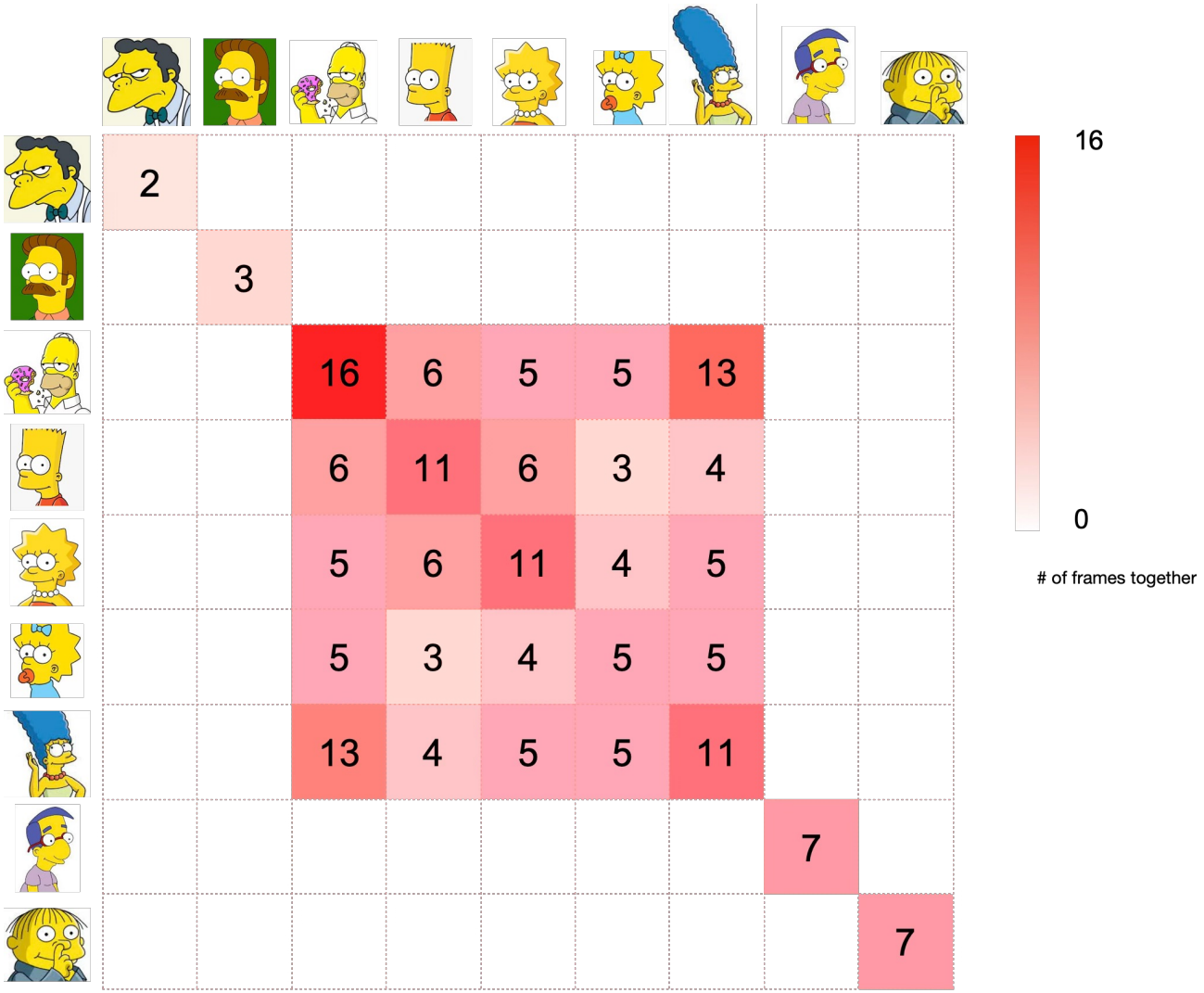
Figs adapted from:
Ulyanov *et al.,* 2015.
Lieberman-Aiden *et al.,* 2009

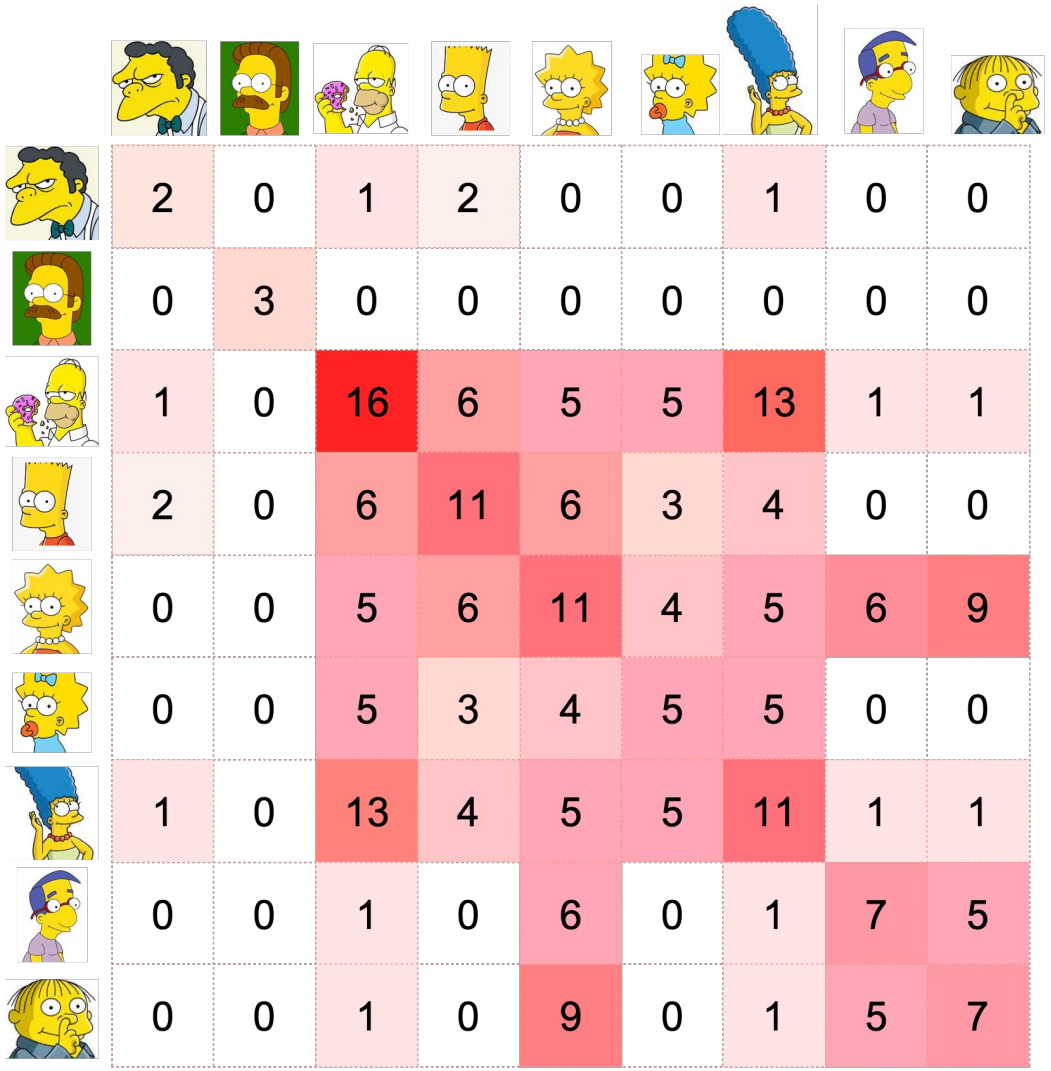
How to read a HiC matrix






2
3
16
11
11
5
11
7
7
16
0
# of frames together

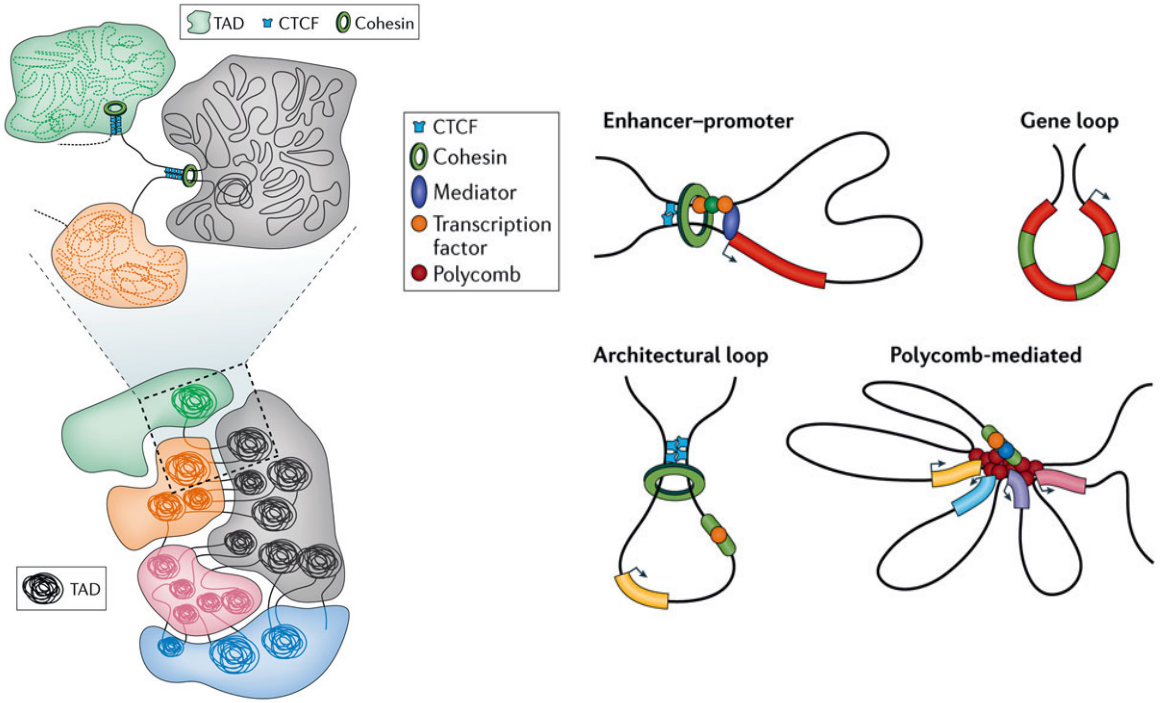

2
3
16 6 5 5 13
6 11 6 3 4
5 6 11 4 5
5 3 4 5 5
13 4 5 5 11
7
7
16
0
# of frames together


2 0 1 2 0 0 1 0 0
0 3 0 0 0 0 0 0 0
1 0 16 6 5 5 13 1 1
2 0 6 11 6 3 4 0 0
0 0 5 6 11 4 5 6 9
0 0 5 3 4 5 5 0 0
1 0 13 4 5 5 11 1 1
0 0 1 0 6 0 1 7 5
0 0 1 0 9 0 1 5 7
16
0
# of frames together

Molecular reconstruction of the 3D genome.

A hierarchical issue

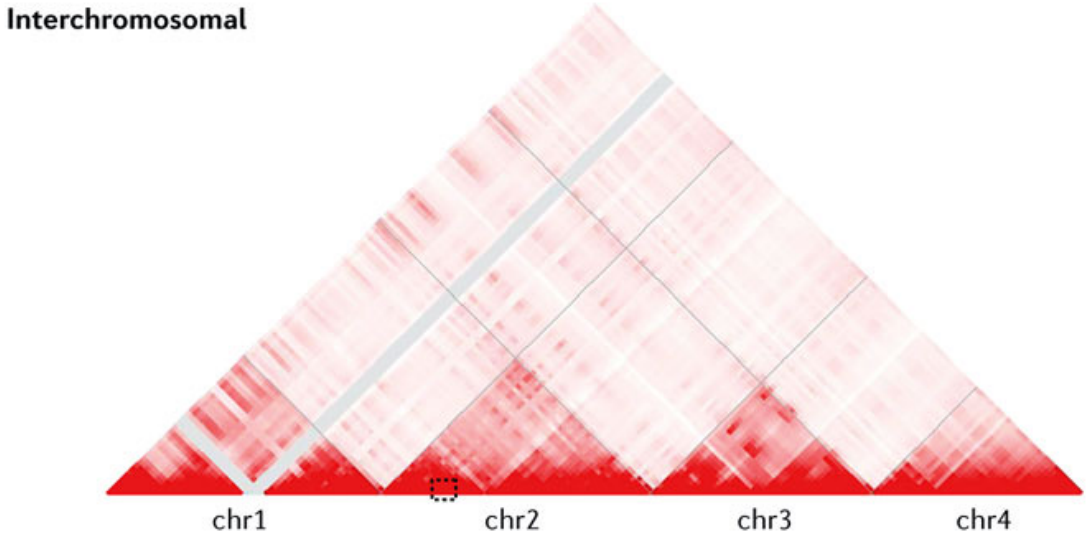
Nature Reviews | Genetics



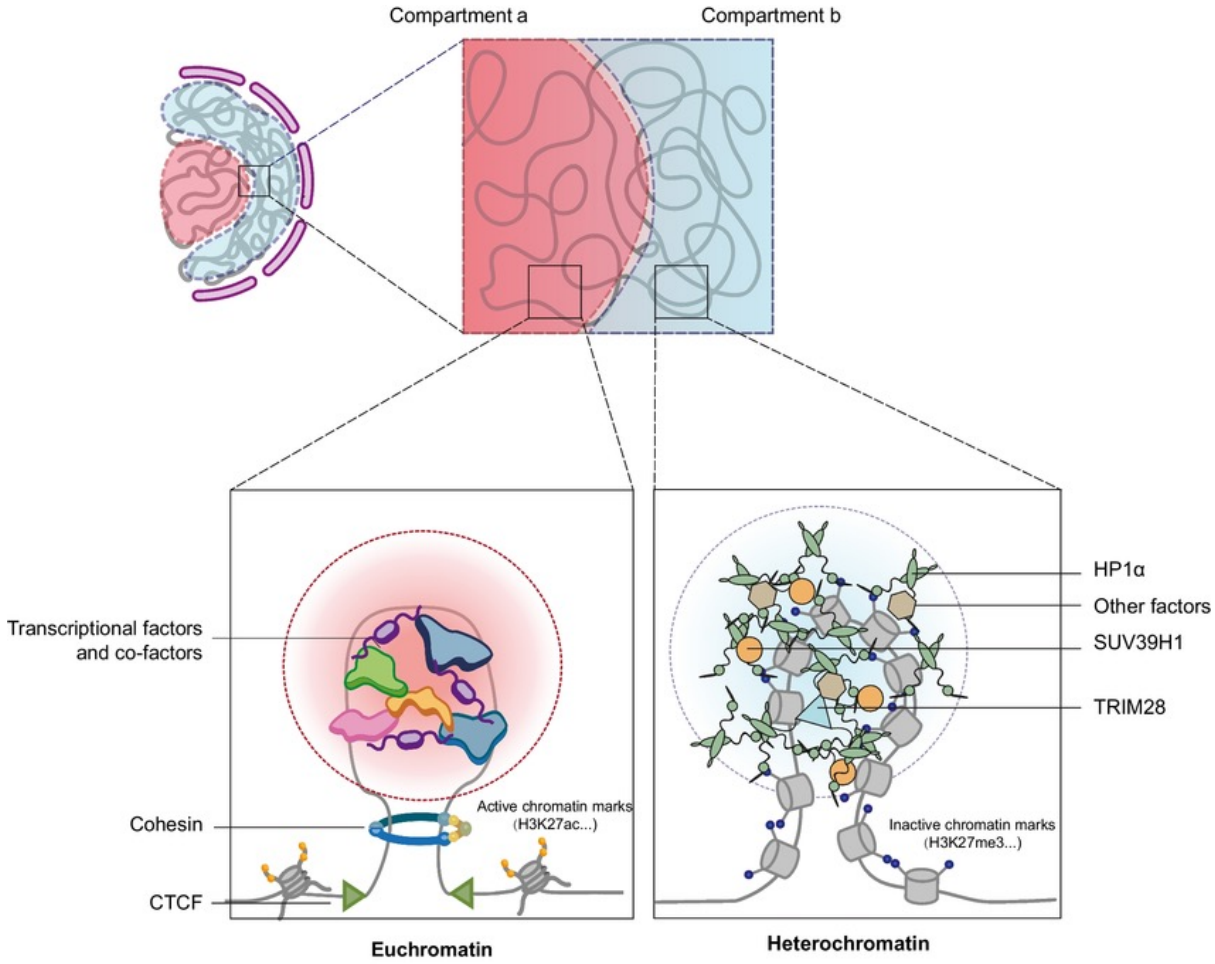
Bonev and Cavalli, *Nat. Rev. Gen,* 2016. https://doi.org/10.1038/nrg.2016.112

Chromosome territories and genome compartmentalization

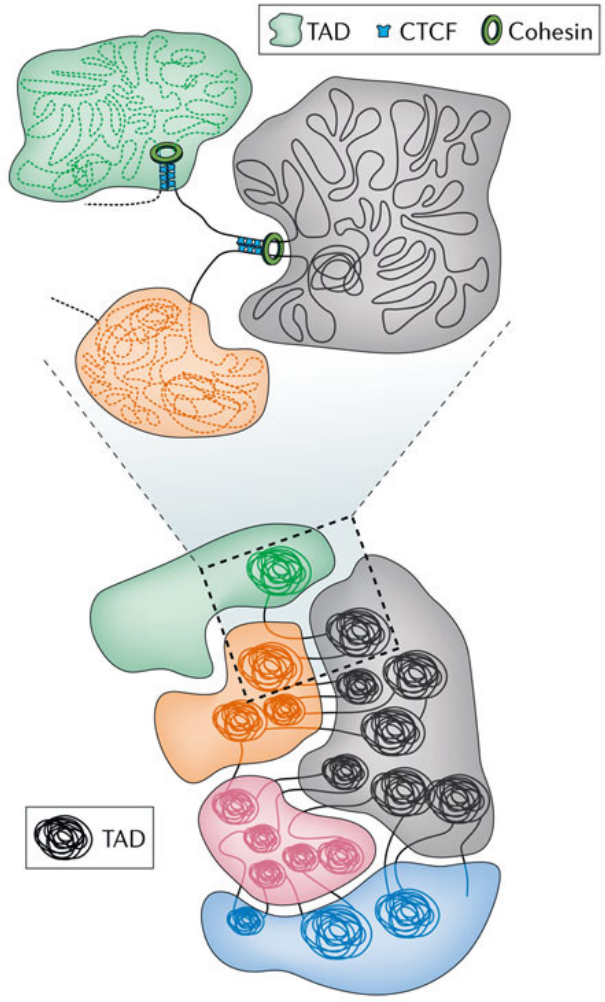
Nature Reviews | Genetics

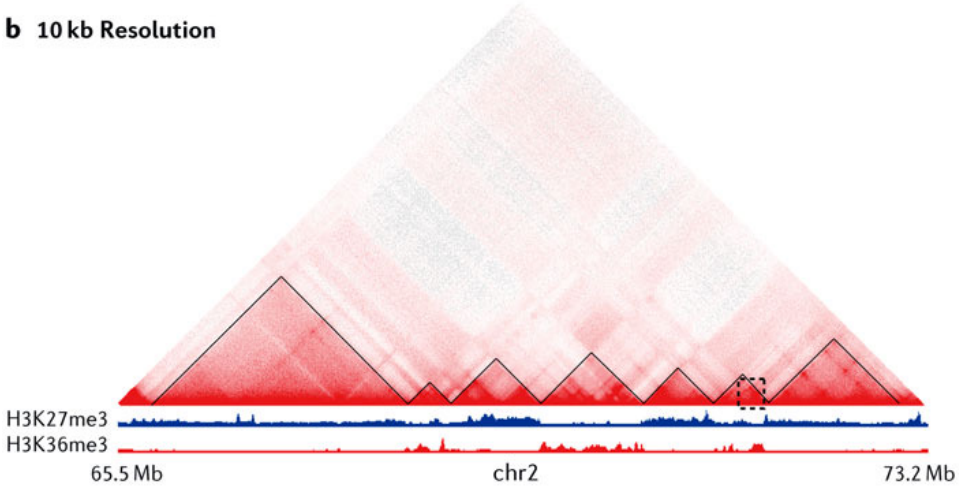


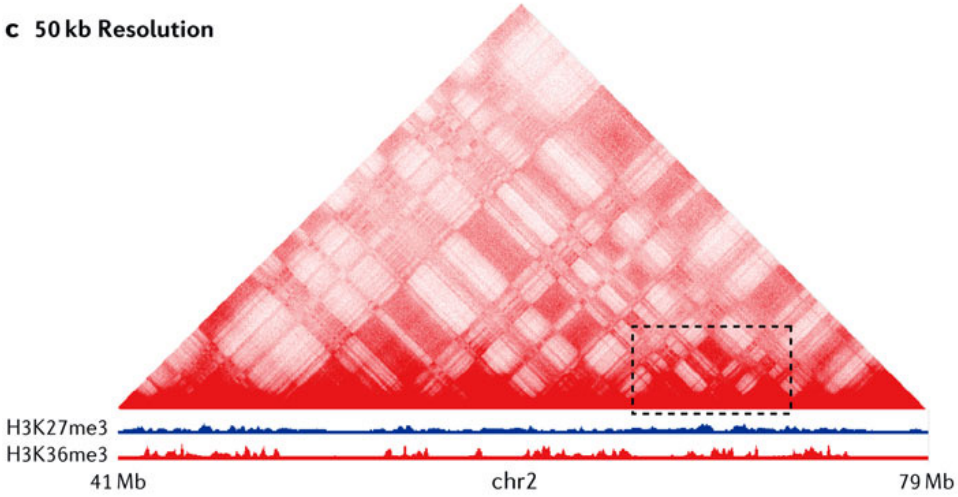
Bonev and Cavalli, *Nat. Rev. Gen,* 2016. https://doi.org/10.1038/nrg.2016.112



Lin *et al*, *Cell and Tissue Research,* 2025. 10.1007/s00441-025-03974-2



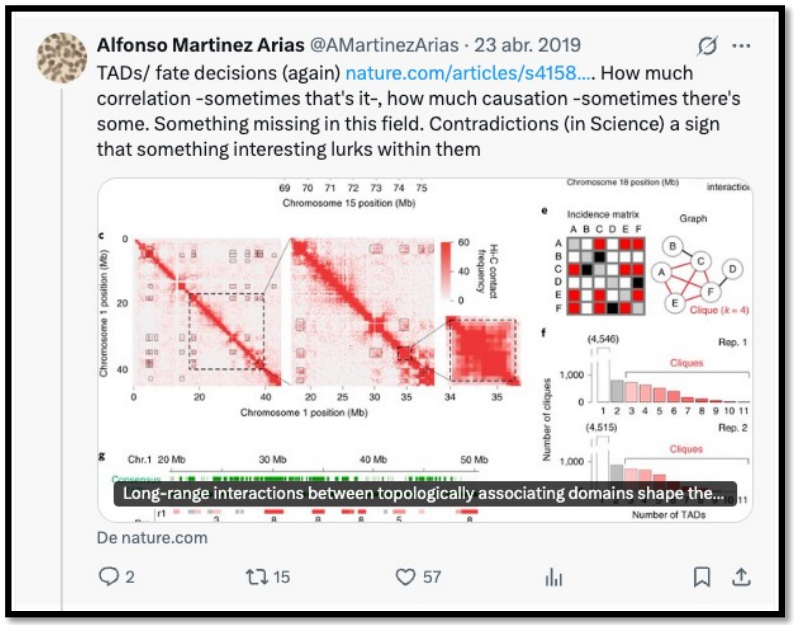


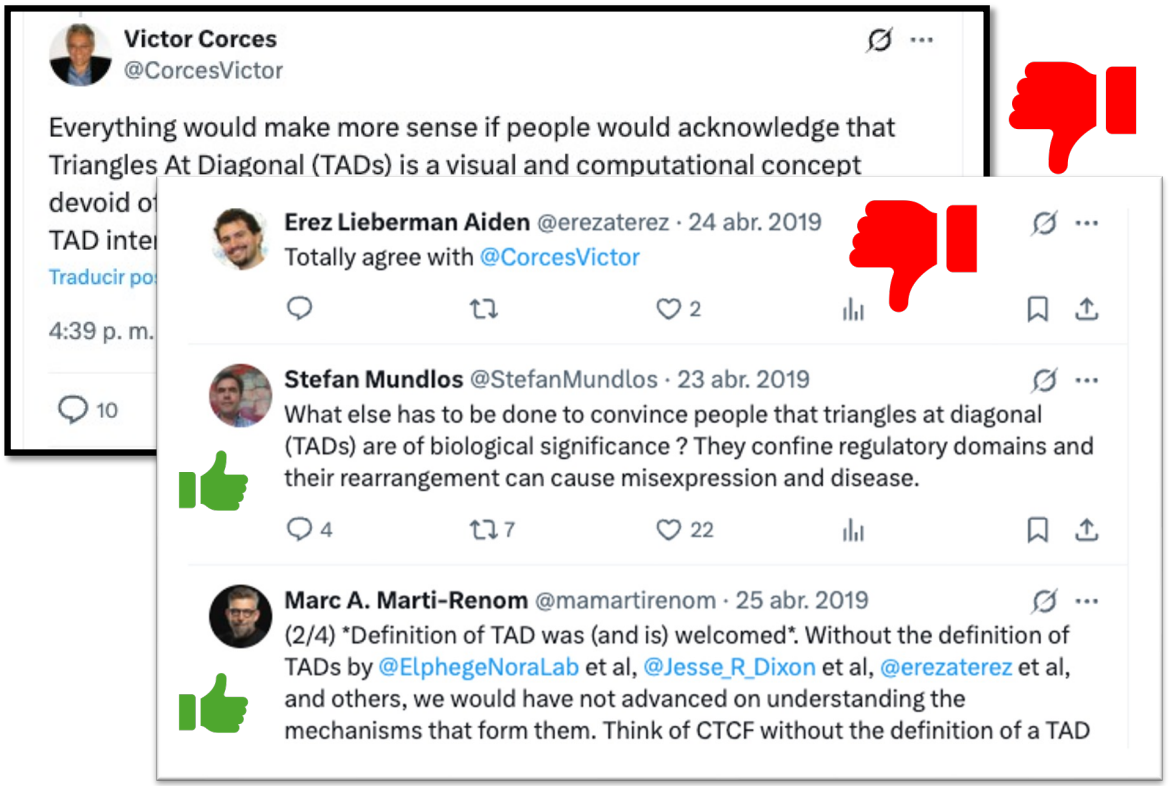


Bonev and Cavalli, *Nat. Rev. Gen,* 2016. https://doi.org/10.1038/nrg.2016.112

TADs are functional units

(Though some just call them Triangles At the Diagonal)

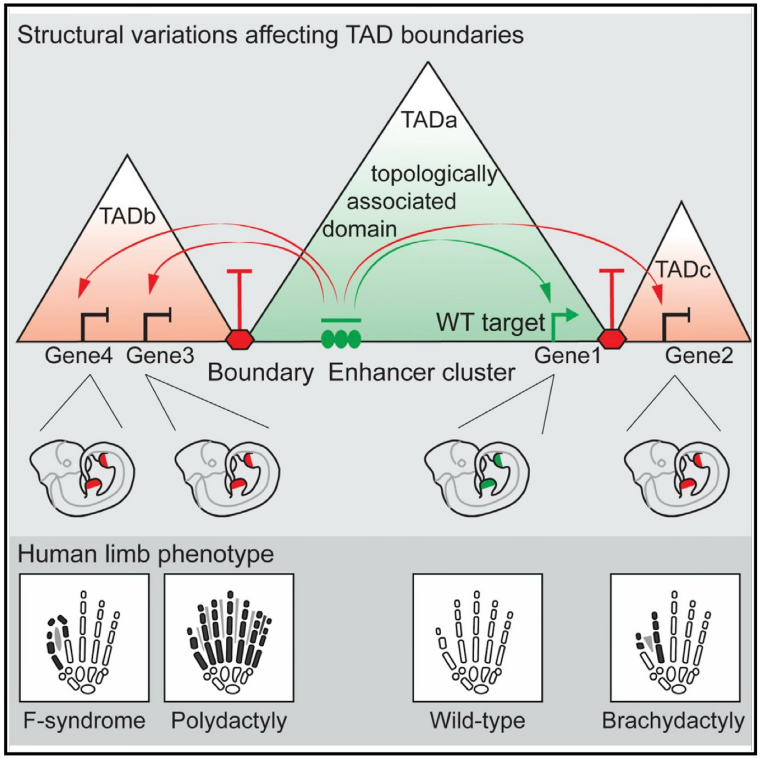


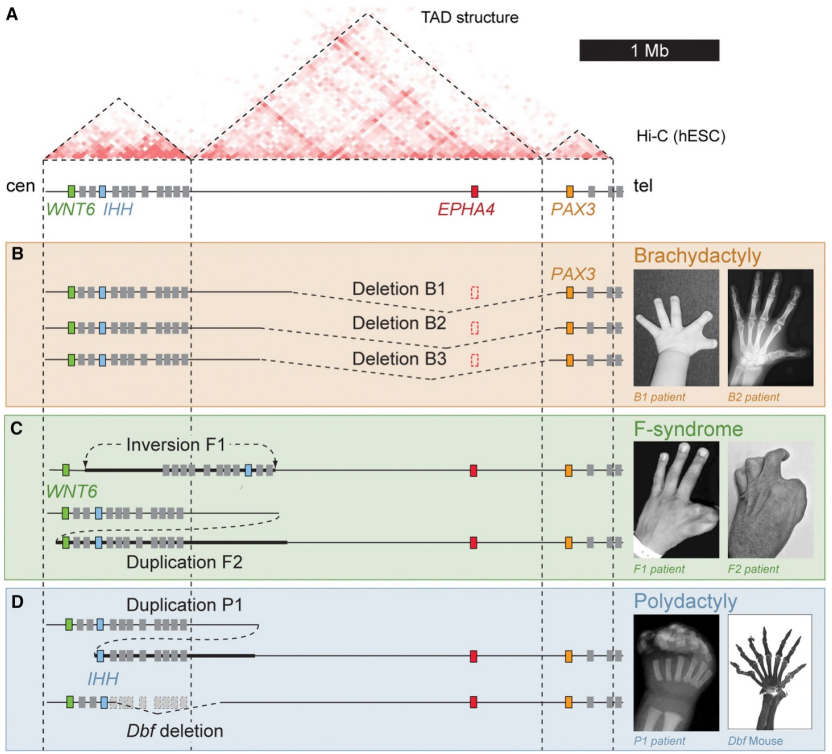


Lupiáñez *et al*, *Cell,* 2015. 10.1016/j.cell.2015.04.004

TADs are functional units

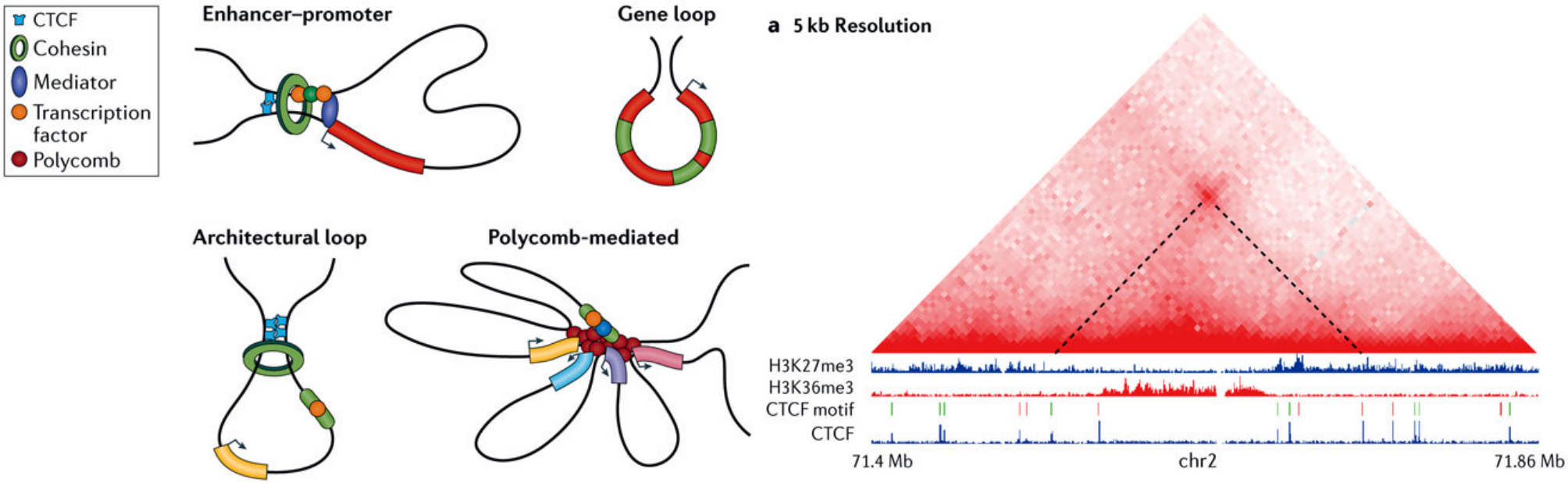
Or are they?



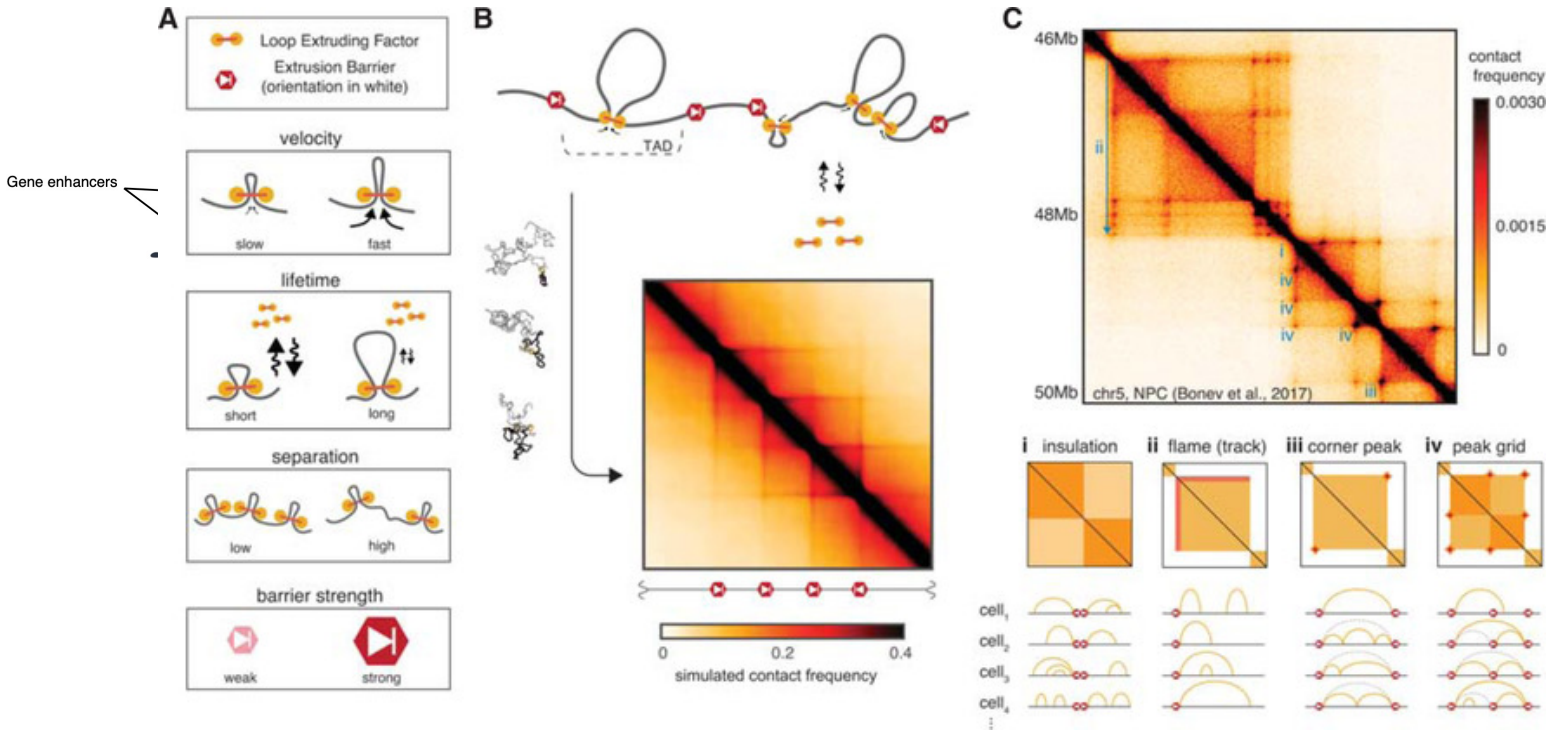


Lupiáñez *et al*, *Cell,* 2015. 10.1016/j.cell.2015.04.004

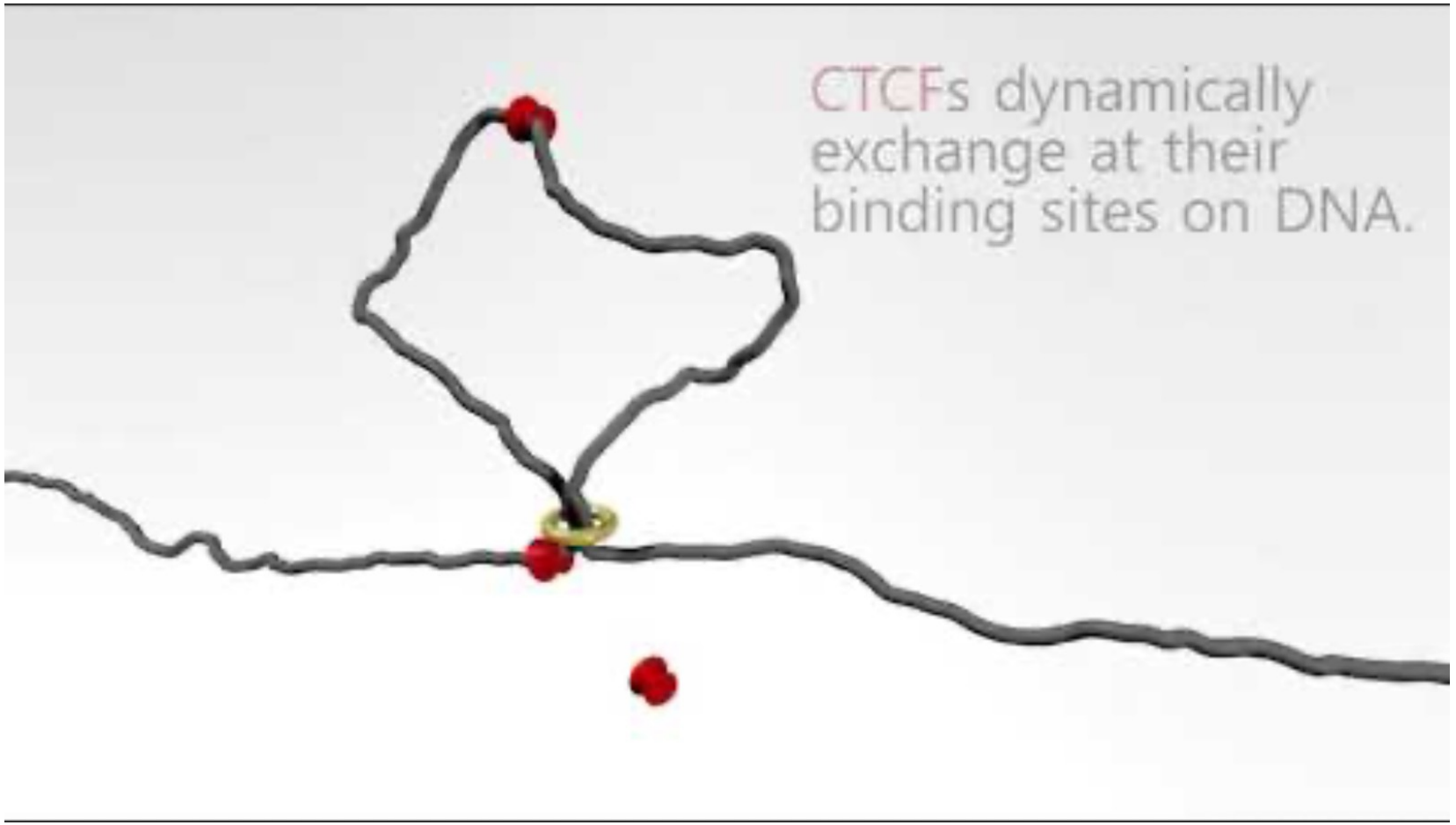
Loops


CTCF
Cohesin
Mediator
Transcription factor
Polycomb
Enhancer–promoter
Gene loop
Architectural loop
Polycomb-mediated
a 5 kb Resolution
H3K27me3
H3K36me3
CTCF motif
CTCF
71.4 Mb
chr2
71.86 Mb

Loop extrusion as a TAD forming mechanism



Fudenberg *et al.,* 2018 Cold Spring Harb Symp Quant Biol 2017 doi: 10.1101/sqb.2017.82.034710

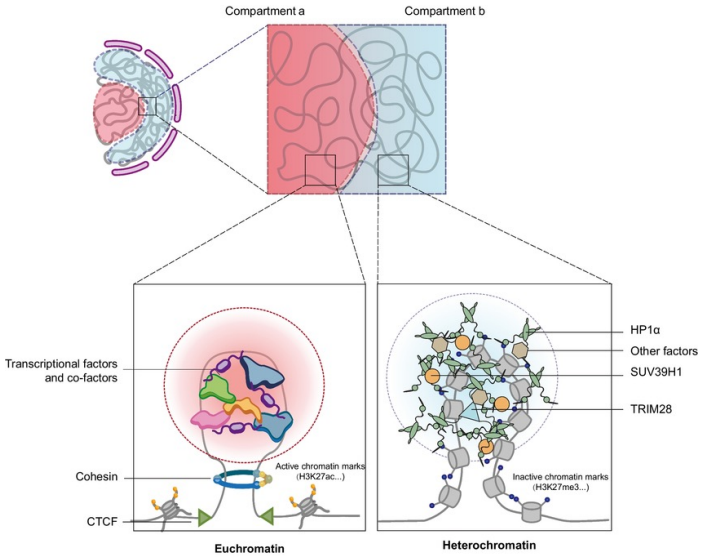


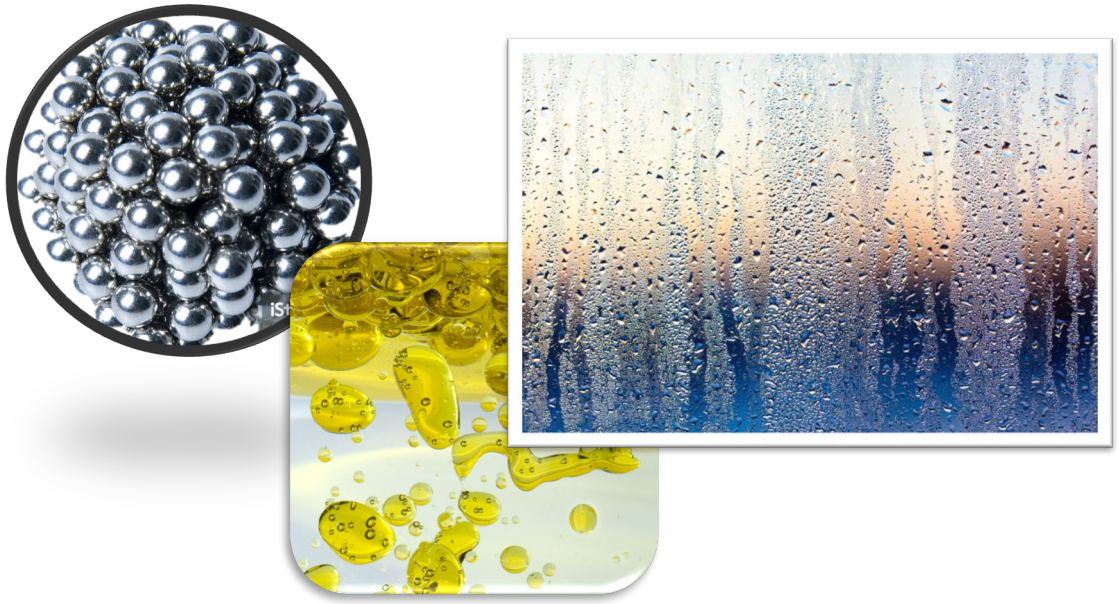
https://www.youtube.com/watch?v=8FW6gOx5lPI

How does the genome look like?

Liquid-liquid phase separation (LLPS)

LLPS is how cells form dynamic, membrane-less compartments by **condensing specific proteins and RNAs into liquid droplets** to regulate biochemical reactions.

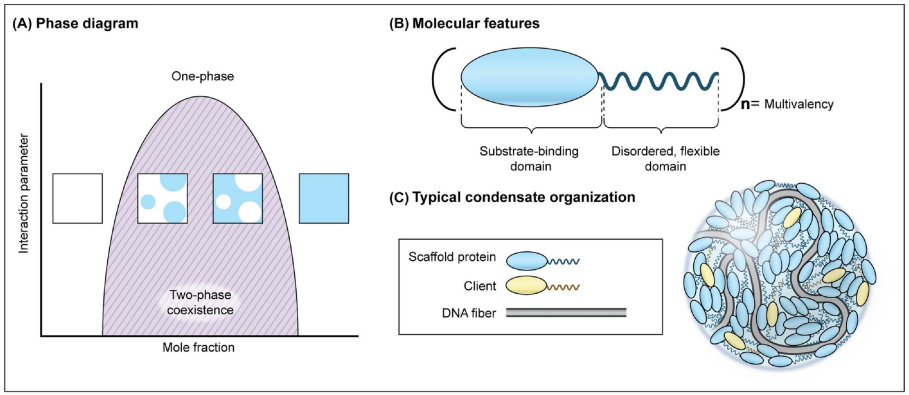


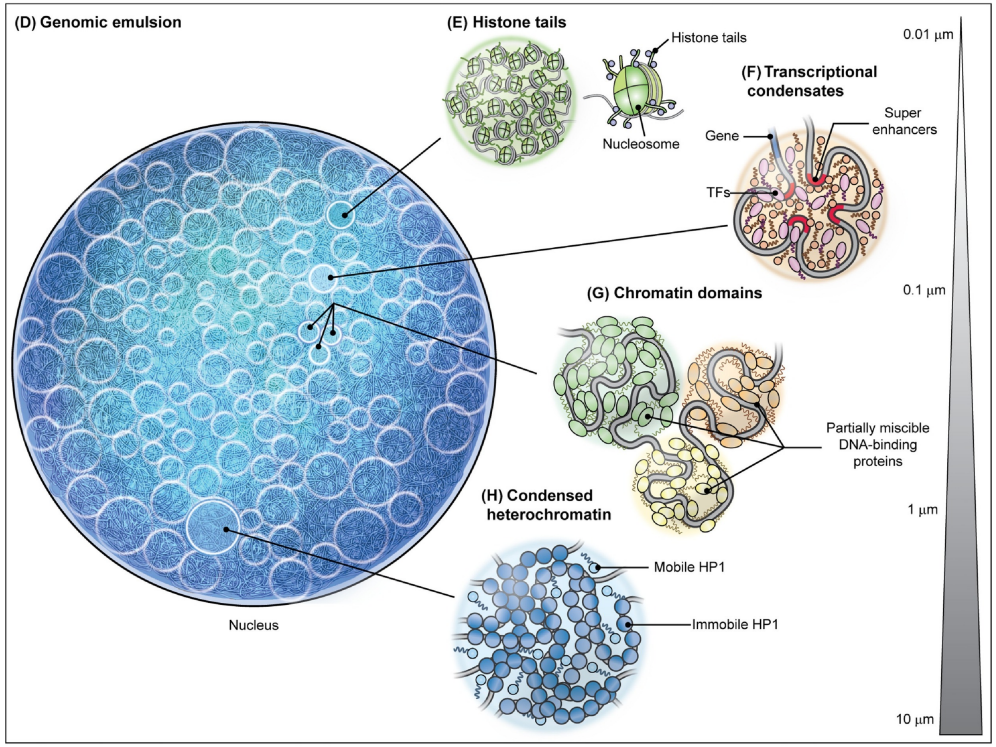


Lin *et al*, *Cell and Tissue Research,* 2025. 10.1007/s00441-025-03974-2
Feric and Misteli, Trends in Cell Biology, 2021. 10.1016/j.tcb.2021.03.001

How does the genome look like?

Liquid-liquid phase separation (LLPS)

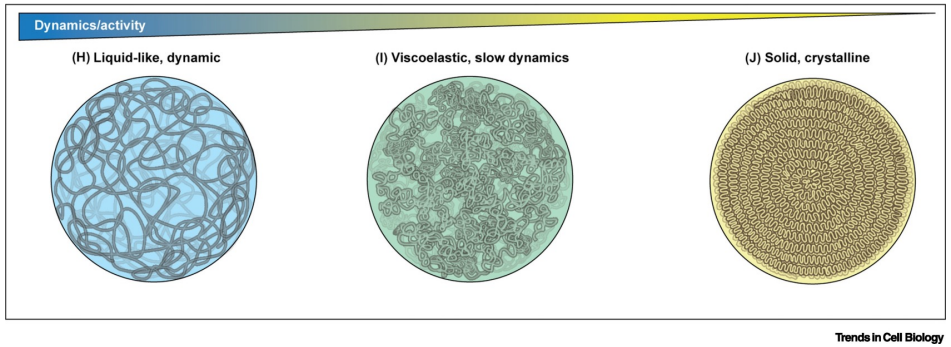


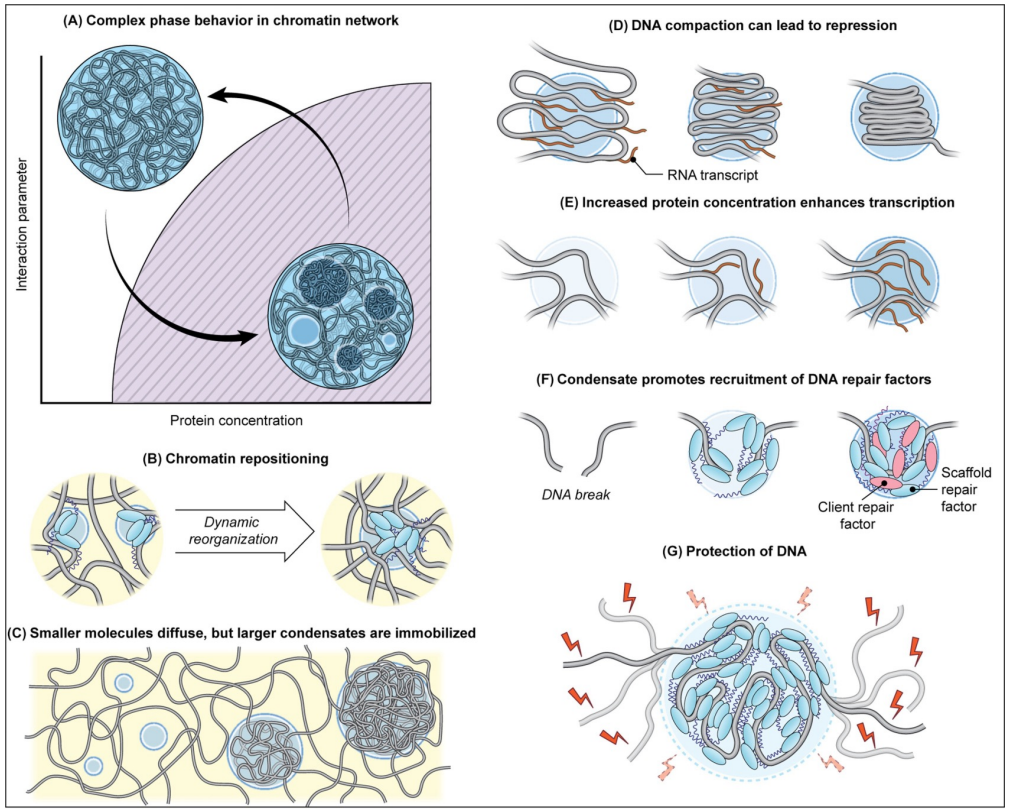


Feric and Misteli, Trends in Cell Biology, 2021. 10.1016/j.tcb.2021.03.001

How does the genome look like?

Liquid-liquid phase separation (LLPS)

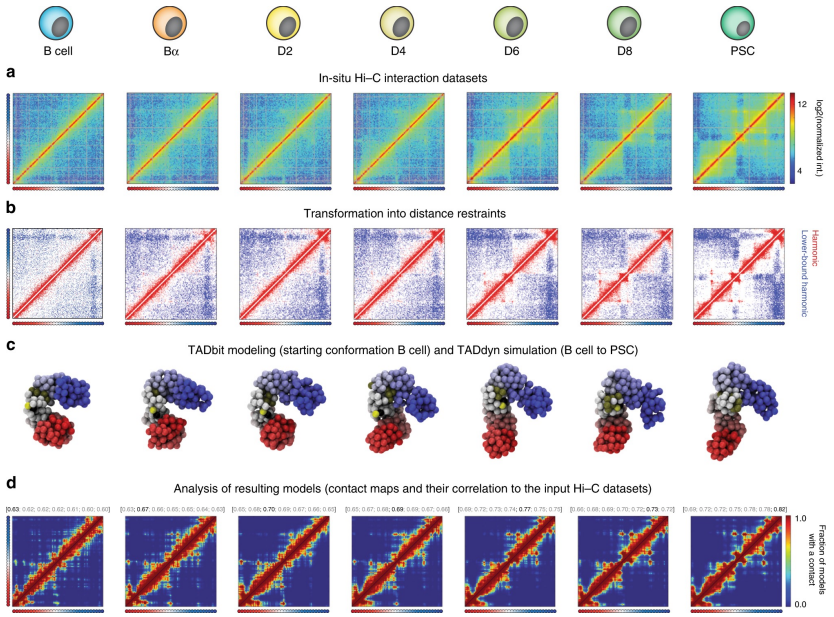




What do we need to know about NGS in HiC

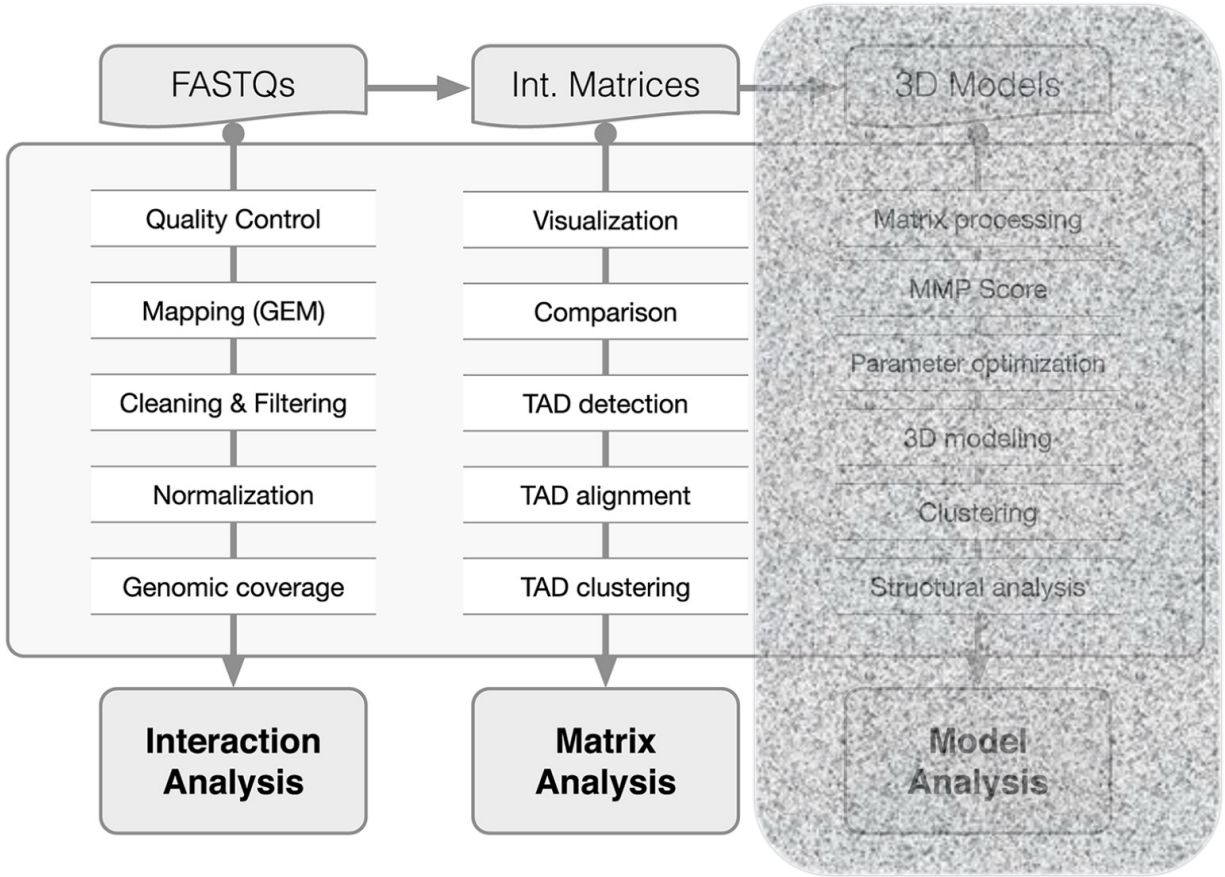
TADbit

a bioinformatic framework to analyse Hi-C experiments



Serra *et al.,* 2017 PLoS Comp Bio
Stadhouders *et al.,* 2018, Nat Gen

TADbit

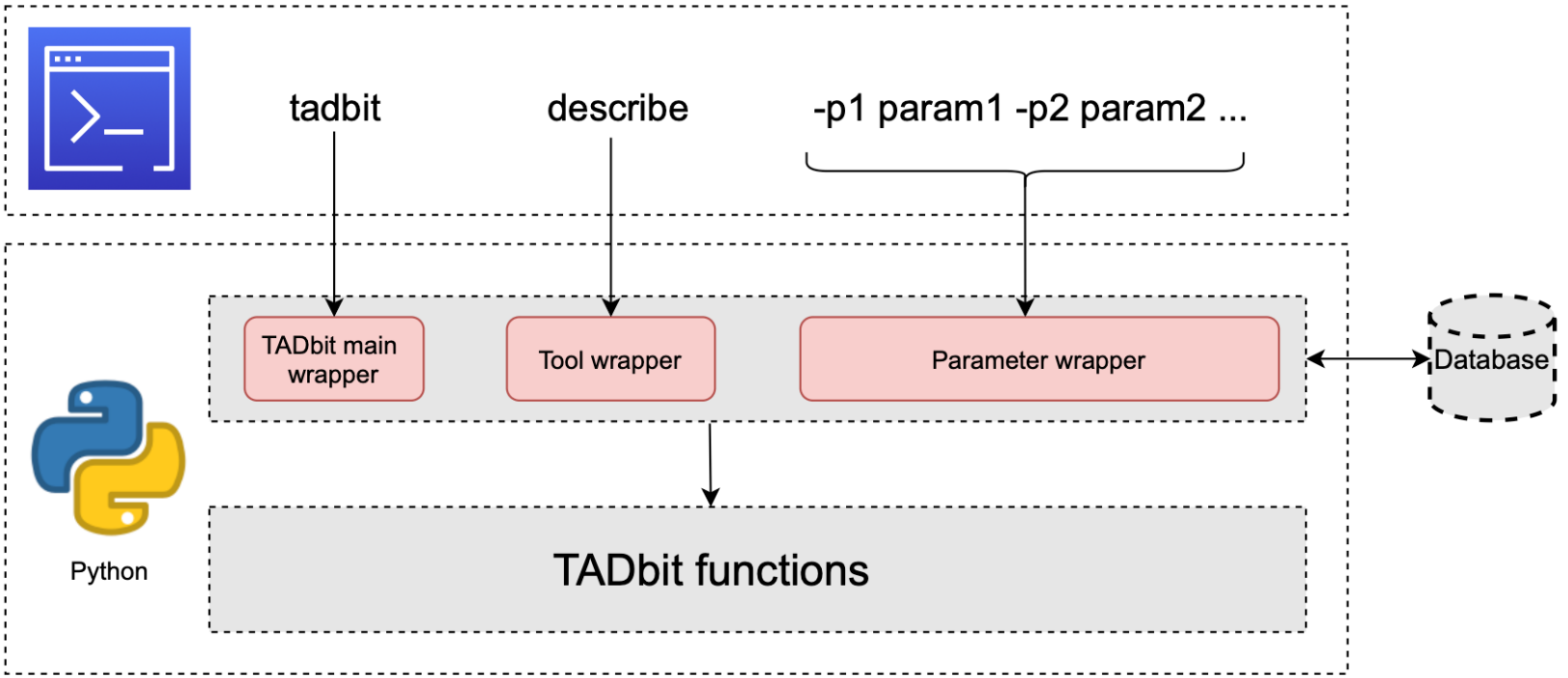

FASTQs
Int. Matrices
3D Models
Quality Control
Mapping (GEM)
Cleaning & Filtering
Normalization
Genomic coverage
Visualization
Comparison
TAD detection
TAD alignment
TAD clustering
Matrix processing
MMP Score
Parameter optimization
3D modeling
Clustering
Structural analysis
Interaction Analysis
Matrix Analysis
Model Analysis

Many alternatives

Tool	Short-read aligner(s)	Mapping improvement	Read filtering	Read-pair filtering	Normalization	Visualization	Confidence estimation	Implementation language(s)
HiCUP [46]	Bowtie/Bowtie2	Pre-truncation	✓	✓	–	–	–	Perl, R
Hiclib [47]	Bowtie2	Iterative	✓[a]	✓	Matrix balancing	✓	–	Python
HiC-inspector [131]	Bowtie	–	✓	✓	–	✓	–	Perl, R
HIPPIE [132]	STAR	✓[b]	✓	✓	–	–	–	Python, Perl, R
HiC-Box [133]	Bowtie2	–	✓	✓	Matrix balancing	✓	–	Python
HiCdat [122]	Subread	–[c]	✓	✓	Three options[d]	✓	–	C++, R
HiC-Pro [134]	Bowtie2	Trimming	✓	✓	Matrix balancing	–	–	Python, R
TADbit [120]	GEM	Iterative	✓	✓	Matrix balancing	✓	–	Python
HOMER [62]	–	–	✓	✓	Two options[e]	✓	✓	Perl, R, Java
Hicpipe [54]	–	–	–	–	Explicit-factor	–	–	Perl, R, C++
HiBrowse [69]	–	–	–	–	–	✓	✓	Web-based
Hi-Corrector [57]	–	–	–	–	Matrix balancing	–	–	ANSI C
GOTHiC [135]	–	–	✓	✓	–	–	✓	R
HiTC [121]	–	–	–	–	Two options[f]	✓	✓	R
chromoR [59]	–	–	–	–	Variance stabilization	–	–	R
HiFive [136]	–	–	✓	✓	Three options[g]	✓	–	Python
Fit-Hi-C [20]	–	–	–	–	–	✓	✓	Python

Analysis methods for studying the 3D architecture of the genome
Ay, F. & Noble, W. S. Genome Biol. 16, 183 (2015).

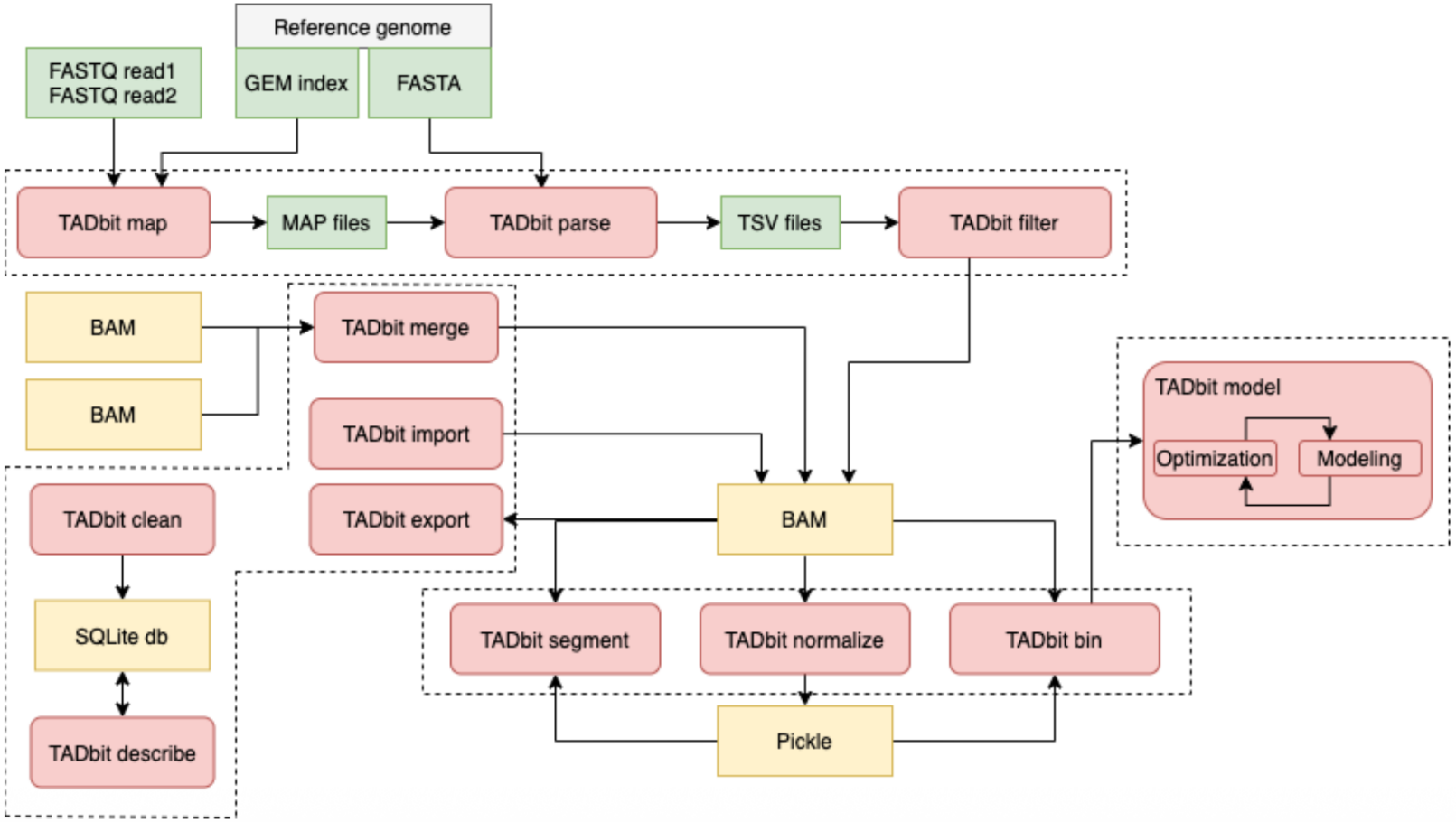
TADbit tools



TADbit tools

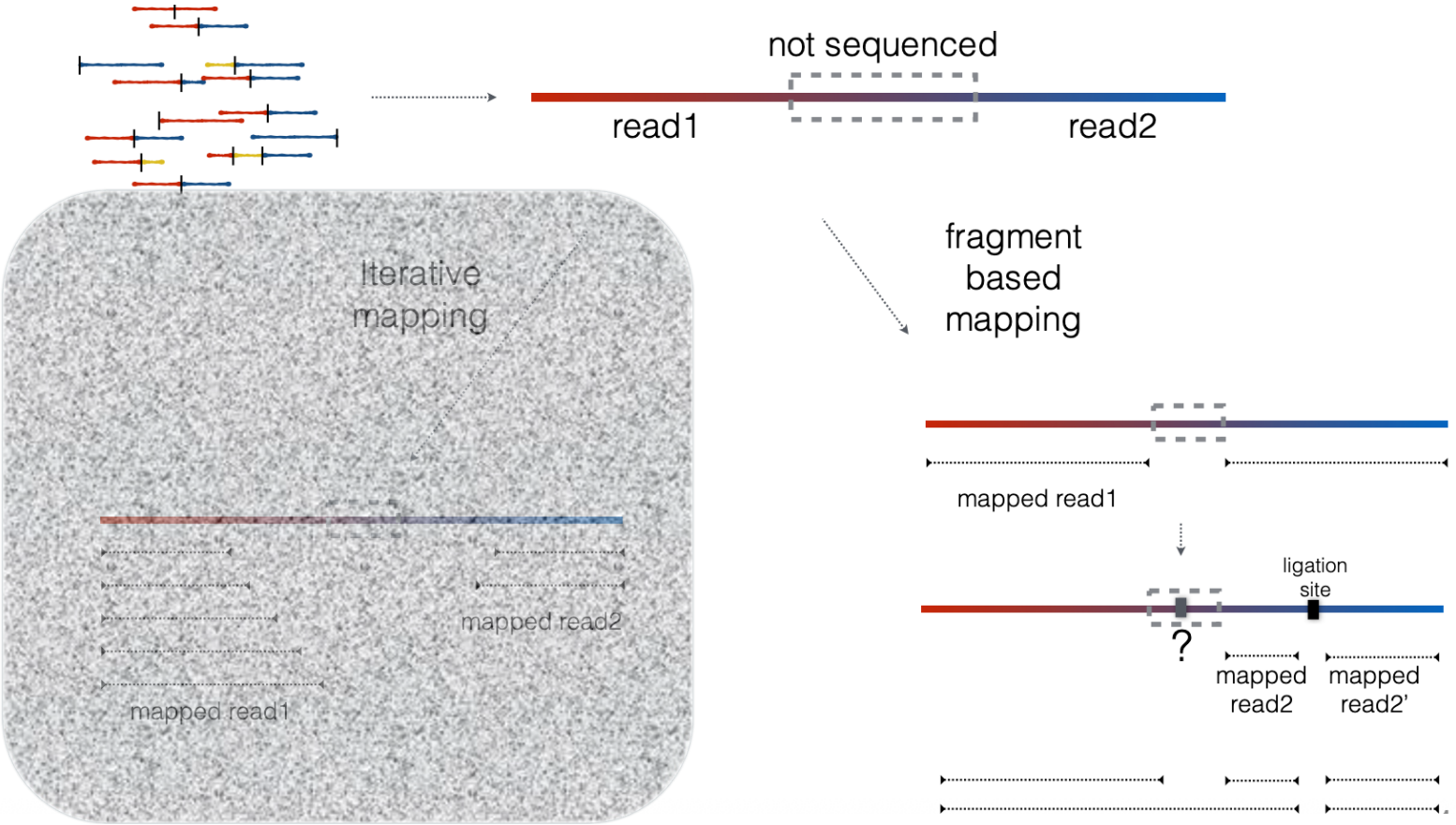
- Appearance of simplicity
- Bioinformaticians are familiar with command line
- The commands can be easily integrated in batch files and pipelines
- The folder structure created automatically when you run the tools is consistent and helps you maintaining an organized environment
- The database helps in the traceability and reproducibility

TADbit tools



> TADbit map

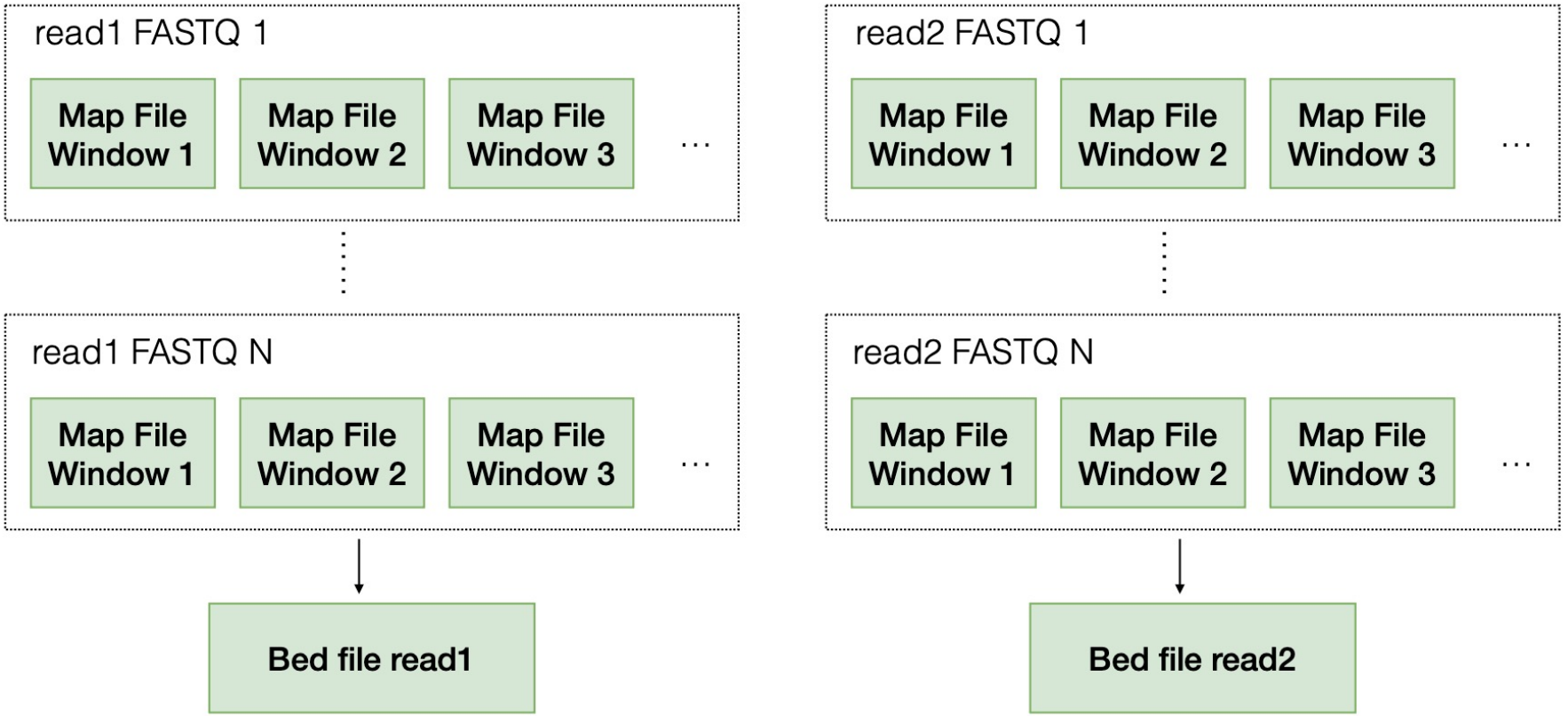
GEM3, bowtie, hisat



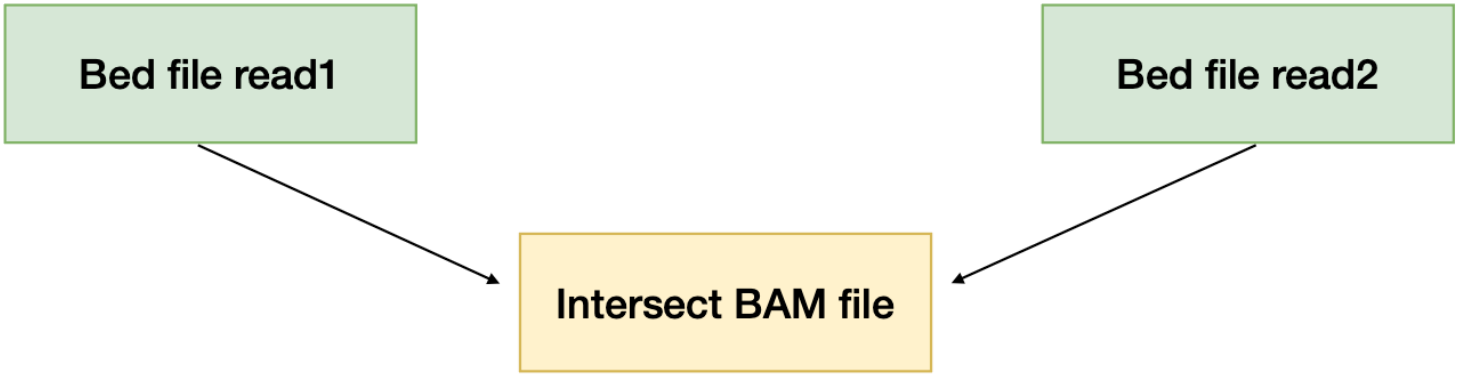
How much do we map?

- 80-90% each end => 64-81% intersection
- 1% multiple contacts
- Many of these will be lost in the filtering…

>TADbit parse

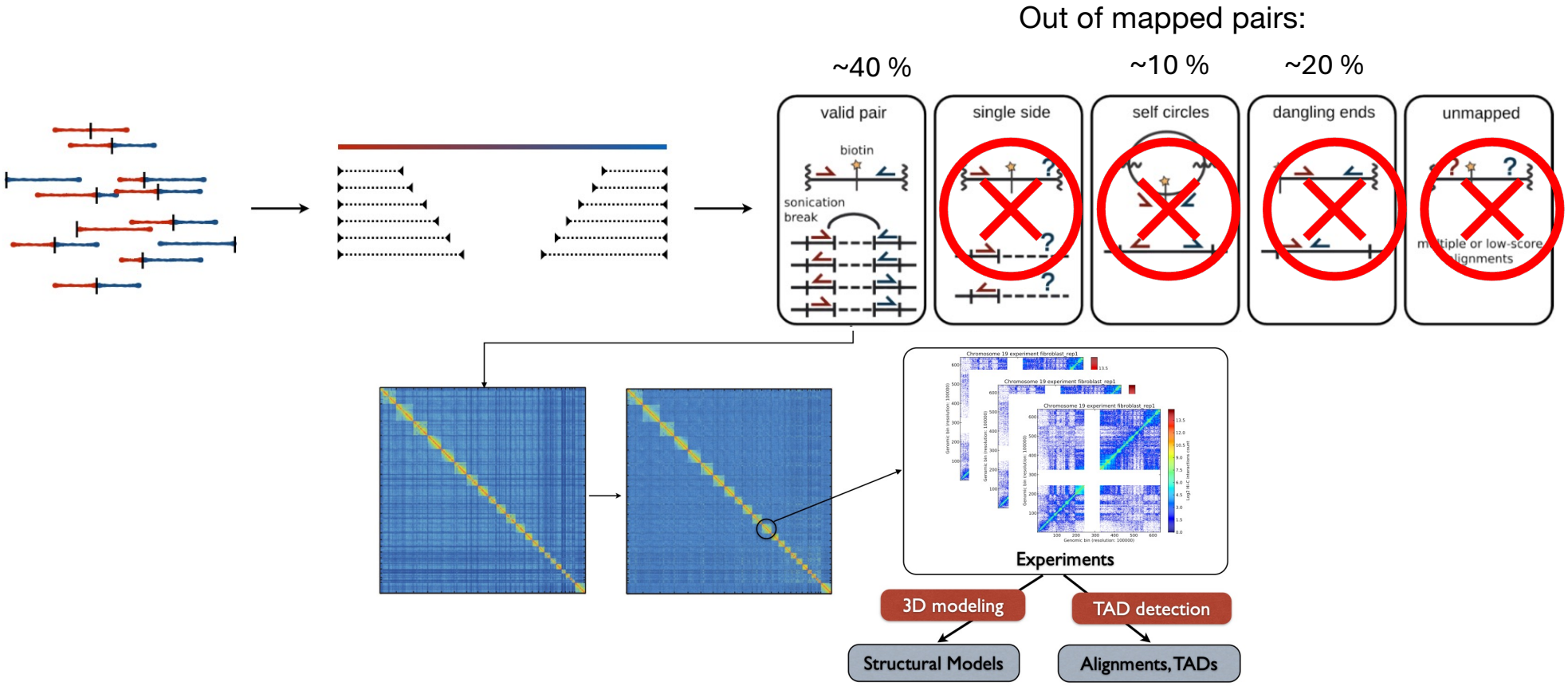


>TADbit parse

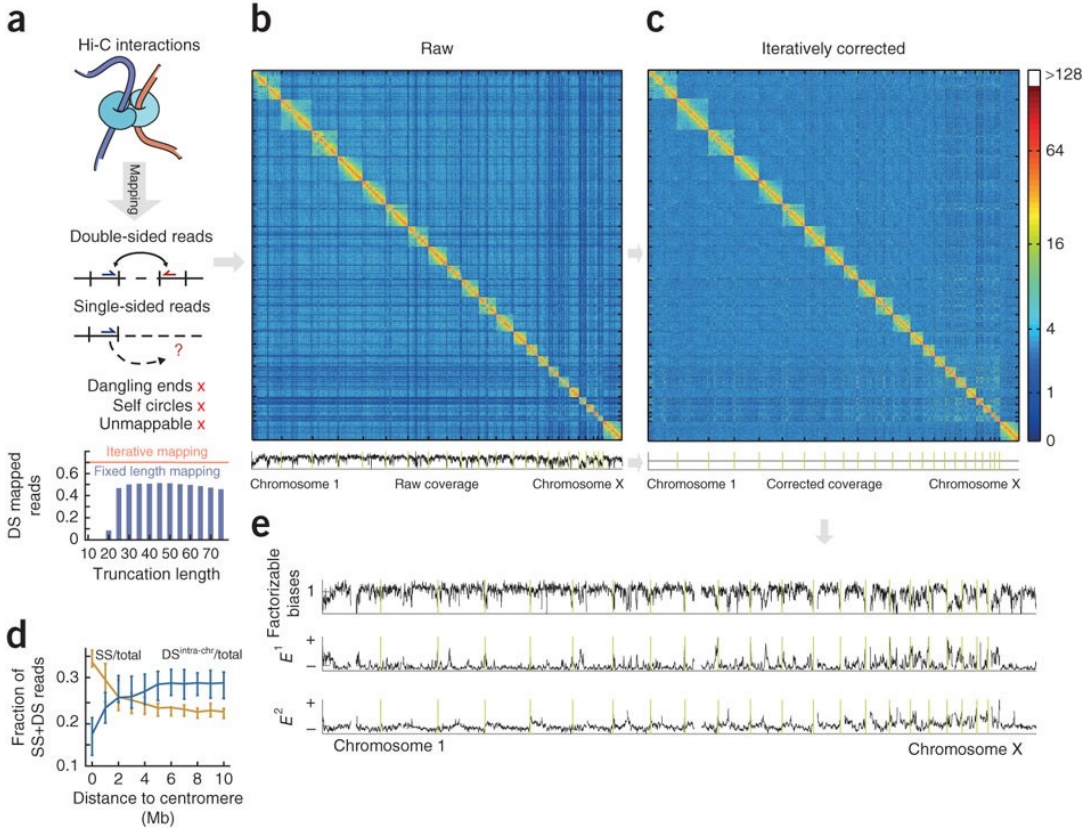


- Paired pseudo BAM (compressed and sorted)
- Each pair is categorised (tagged)
- Mirrored for fast access read1-read2 or read2-read1

>TADbit filter



>TADbit normalize



TADbit tools