Topologically associating domains of chromatin: methods and tools for calling

Part 1

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[1]The Dobzhansky Center for Genome Bioinformatics
St. Petersburg State University

Group meeting at BI

Outline

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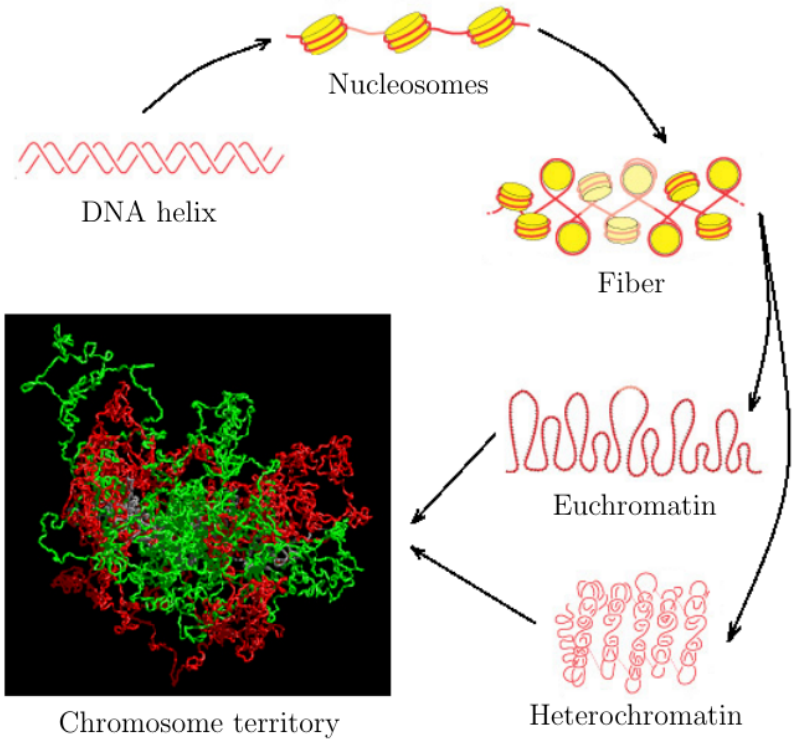
Introduction



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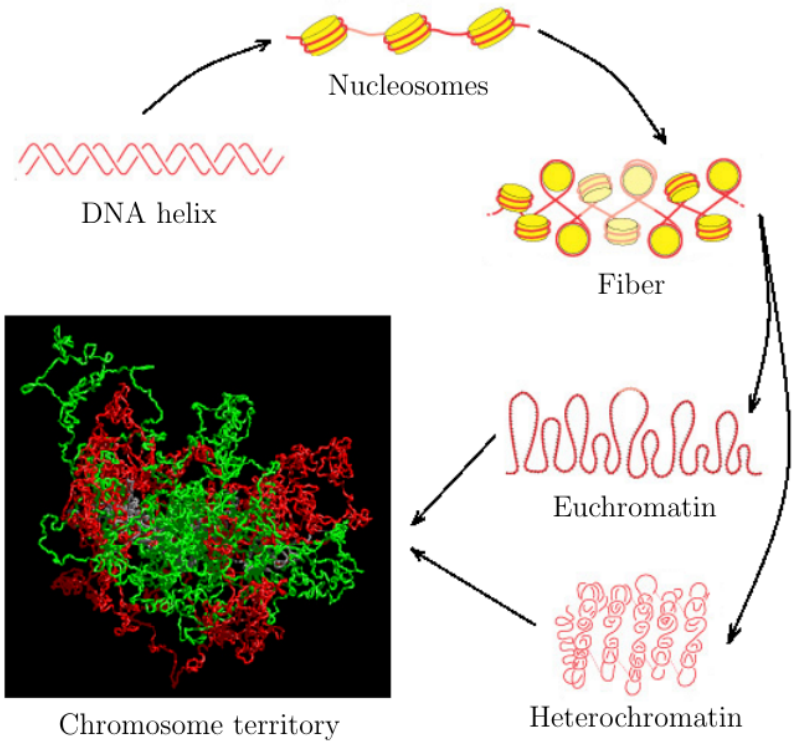

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- **Question:** How is chromatin folded within euchromatin and heterochromatin compartments?

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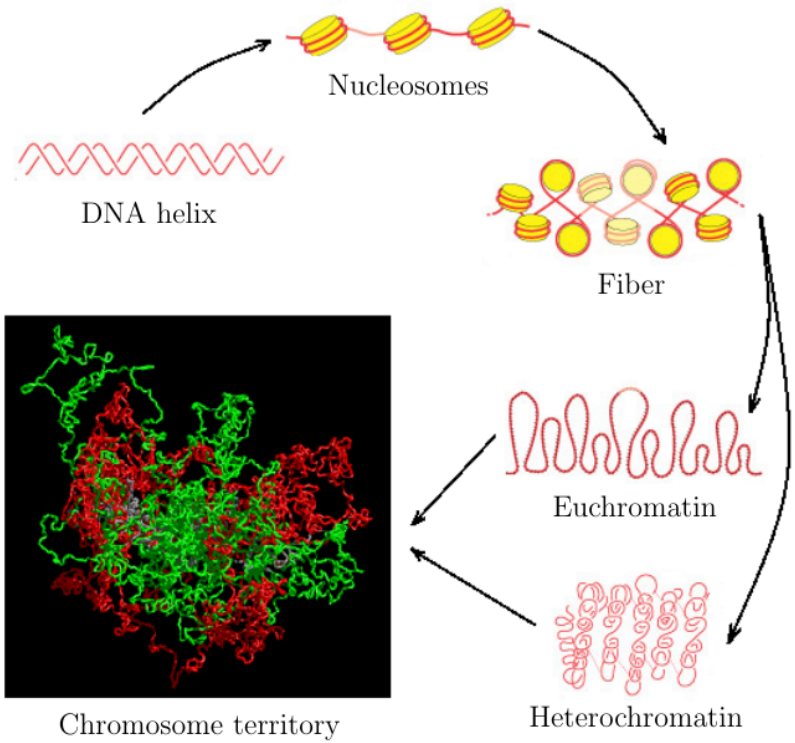

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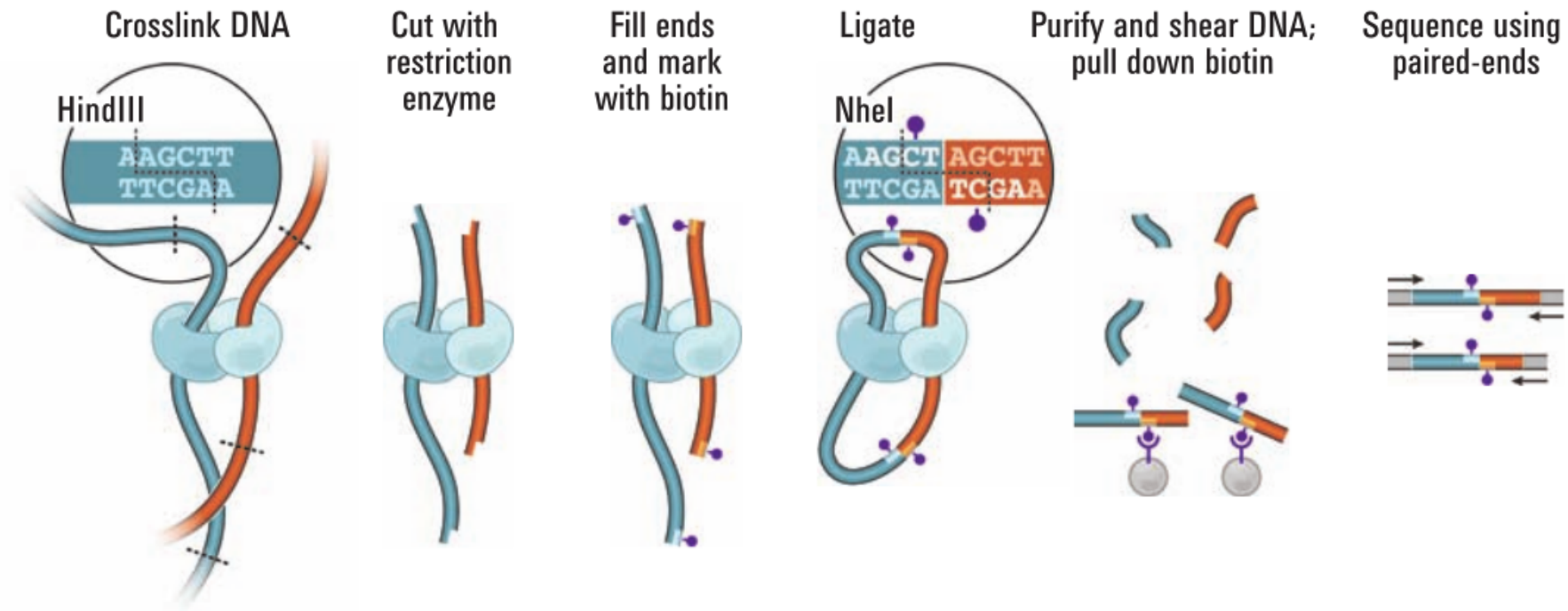
- **Question:** How is chromatin folded within euchromatin and heterochromatin compartments?
- **The answer** came with the development of chromatin conformation capture methods (3C, 2002; 4C, 2006; 5C, 2006; **Hi-C**, 2009).

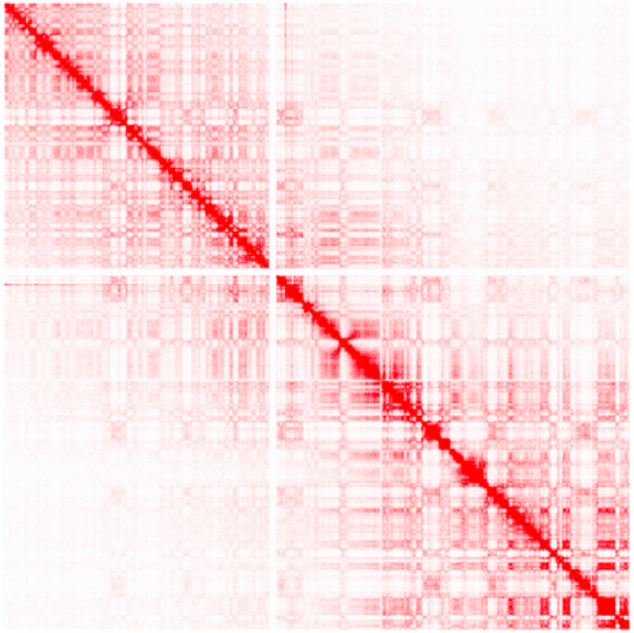
Introduction

Hi-C experiment scheme:



Lieberman-Aiden et al., 2009

Introduction



HOMER tool website

- Chromosome is split into r bp bins (r is called **contact matrix resolution**).
- **Contact matrix** C is built: $C(i, j) \equiv C(j, i)$ is a number of paired-end reads such that one read was mapped into bin i and the other read was mapped into bin j. Contact matrix is usually represented as a heatmap.

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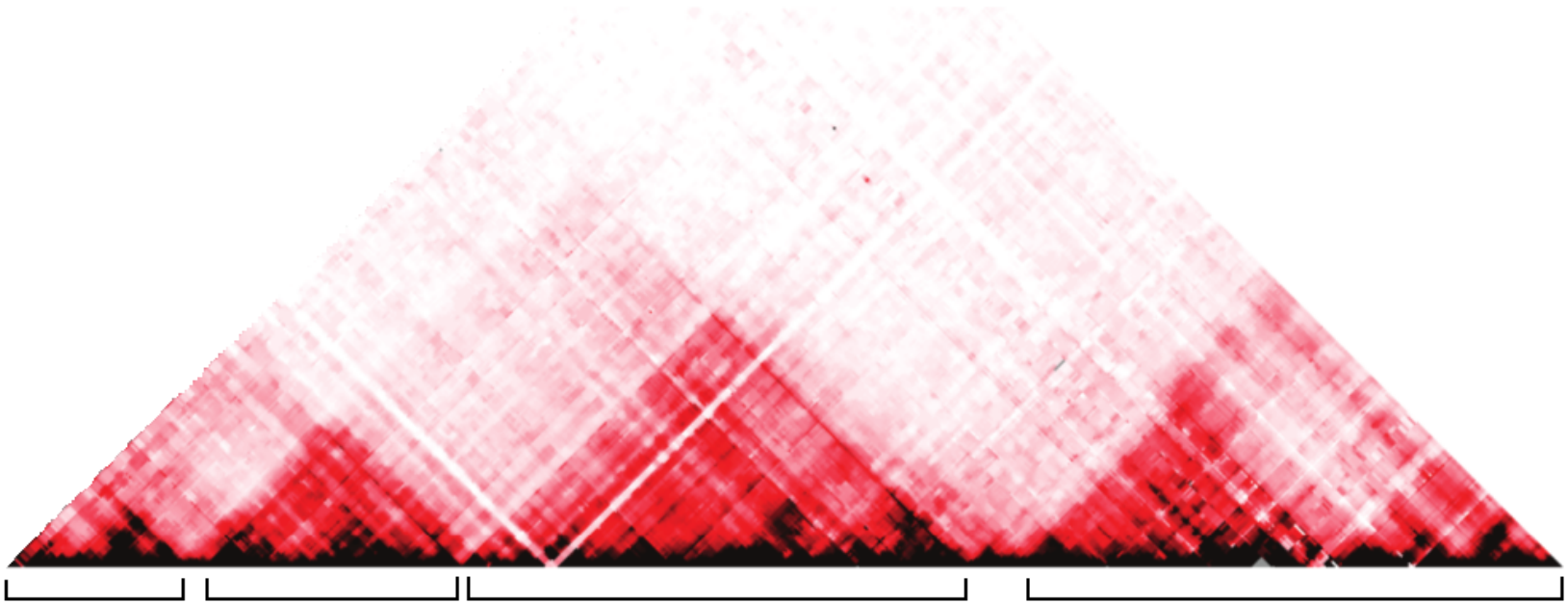
Topologically associating domains



Self-interacting domains can be seen on the main diagonal of a contact matrix (Dekker et al., 2013, adapted).

Topologically associating domains

- Dixon et al., 2012 found self-interacting domains in human and mouse using Hi-C data.

LETTER

doi:10.1038/nature11082

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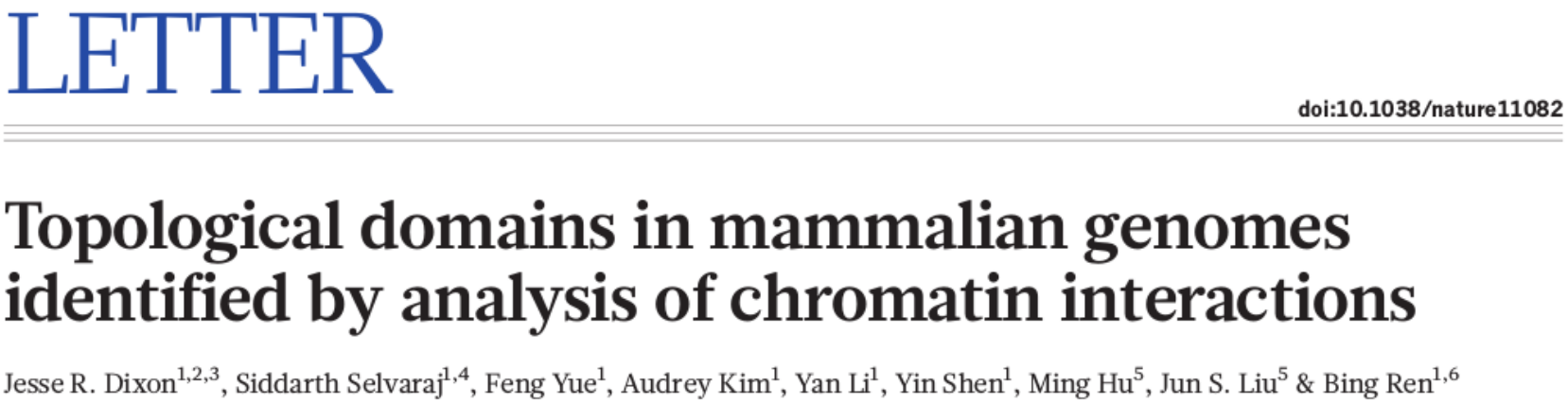

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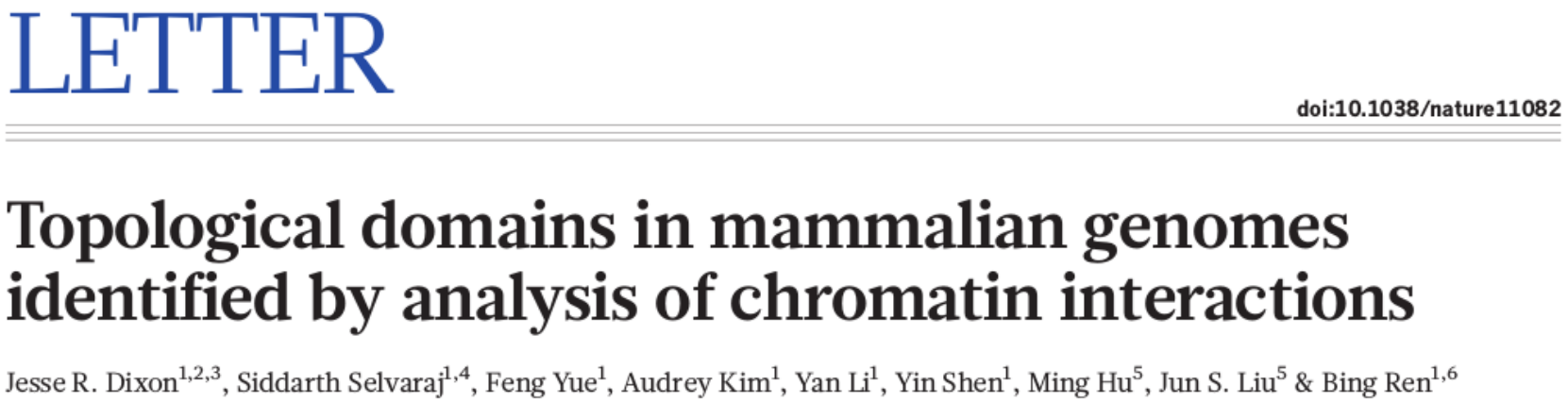

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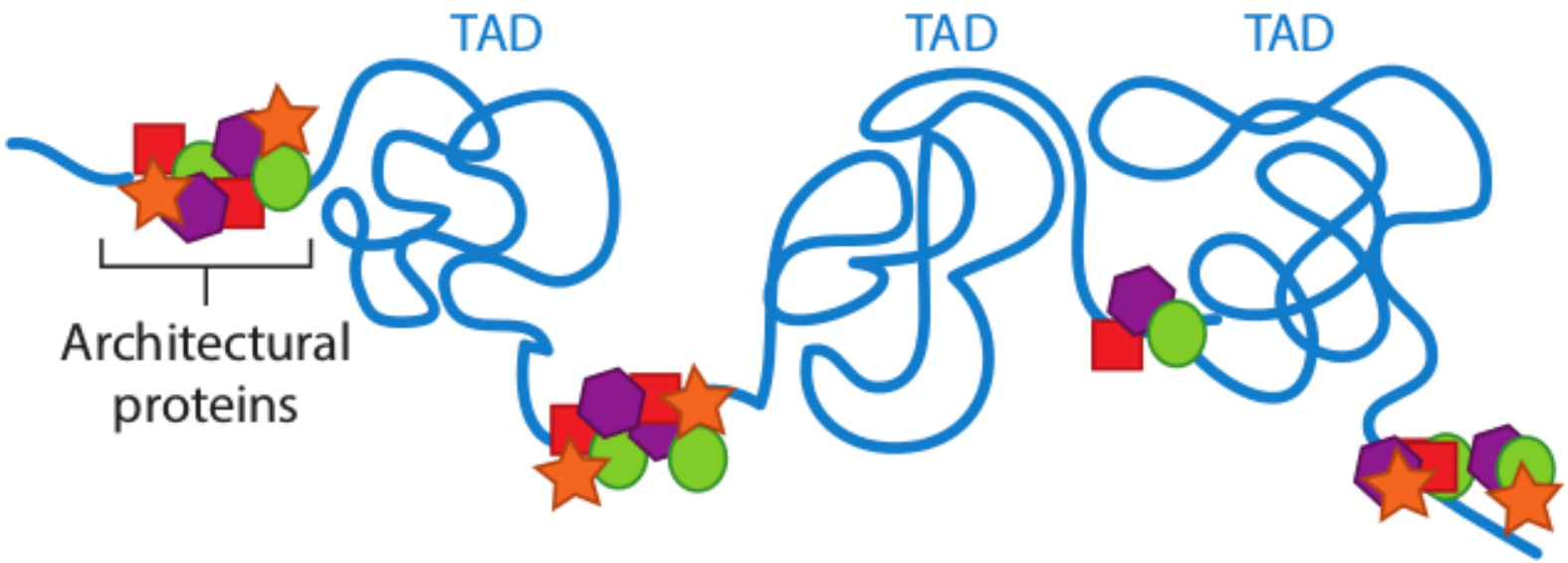
- Similar domains were found in *Drosophila* genome in the same year: Sexton et al., 2012; Hou et al., 2012.
- TADs were also found in the same year in mouse X chromosome by Nora et al., 2012.

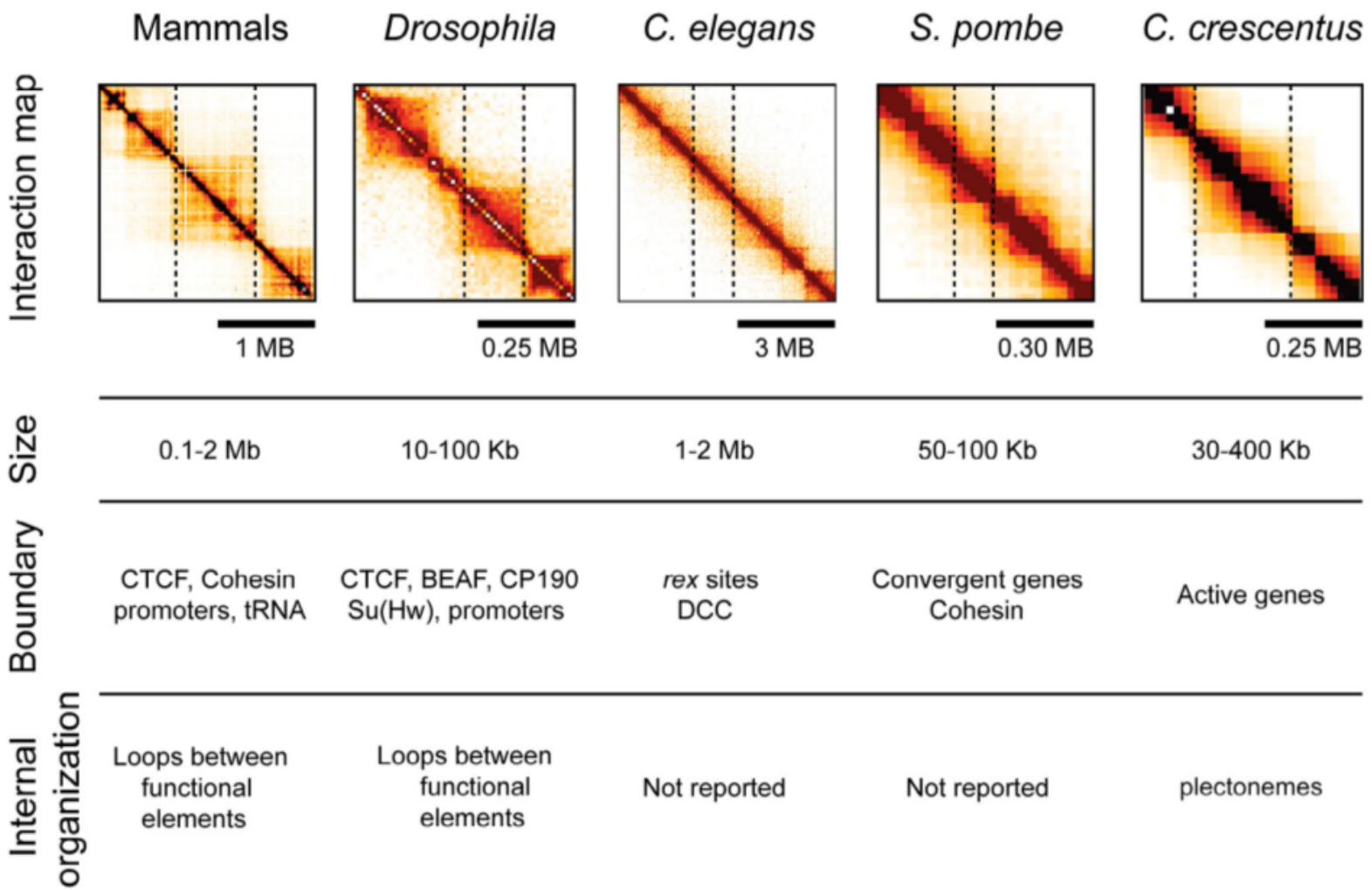
Topologically associating domains



Nguyen H. G. and Bosco G., 2015

- TADs are collections of many chromatin loops.
- TADs are separated by **TAD borders** (intervening chromatin).
- Mammalian TAD borders are enriched in active transcription, housekeeping genes, tRNA genes and SINE repeats, as well as binding sites for the architectural proteins CTCF and cohesin (Dekker J. and Heard E., 2015).

Topologically associating domains



TAD-like domains were found in several organisms in 2012 – 2015 (Dekker J. and Heard E., 2015, adapted).

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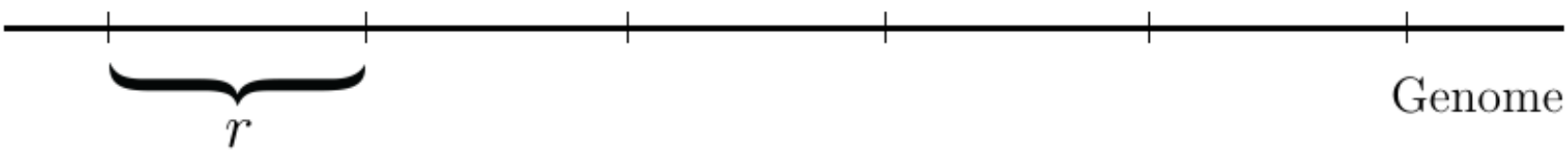
Self-interacting domains in other organisms can have different functions (Dekker J. and Heard E., 2015).

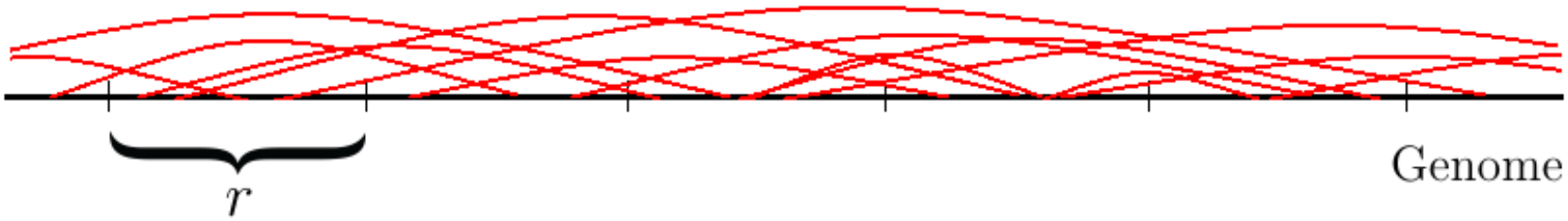
Outline

Directionality index



- Let's partition each chromosome into *r* bp bins, where *r* is a contact matrix resolution.

Directionality index



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- Contacts within the chromosome can then be visualized like this. Each arc denotes a pair of reads.

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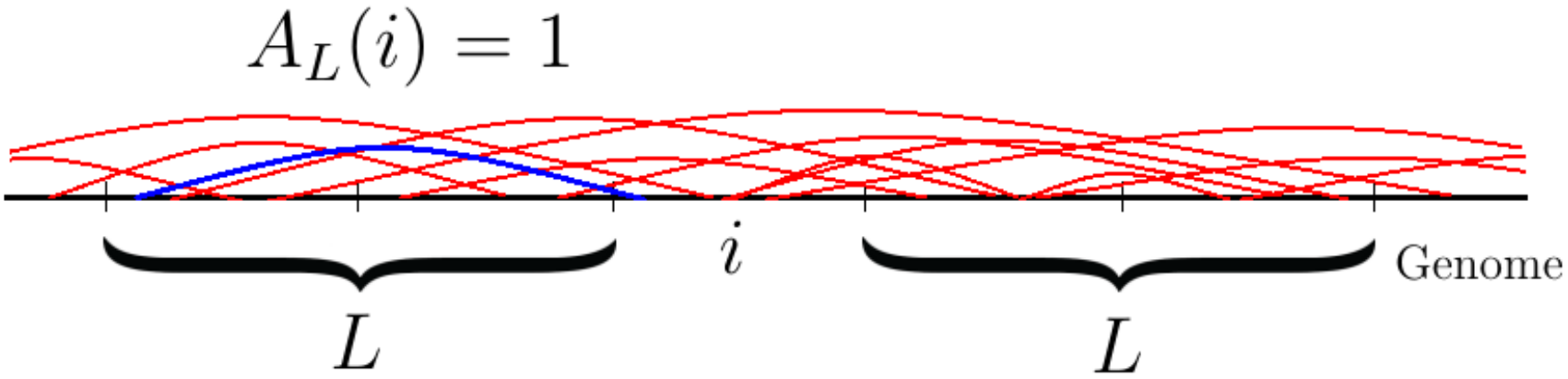

- Let's partition each chromosome into r bp bins, where r is a contact matrix resolution.
- Contacts within the chromosome can then be visualized like this. Each arc denotes a pair of reads.
- Then $A_L(i)$ is the number of read pairs that map from the bin i to the upstream L bp. L should be a multiple of r.

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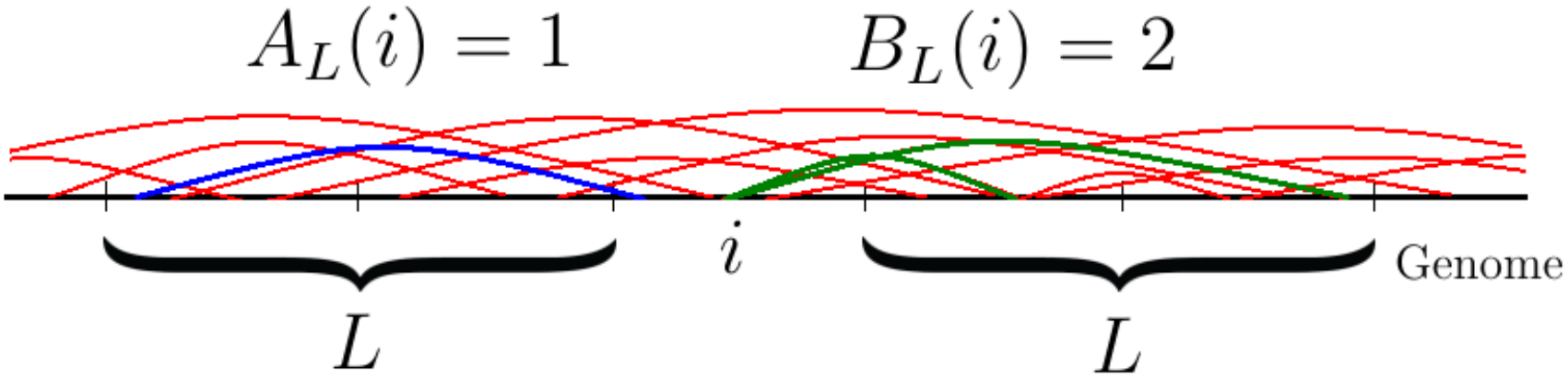
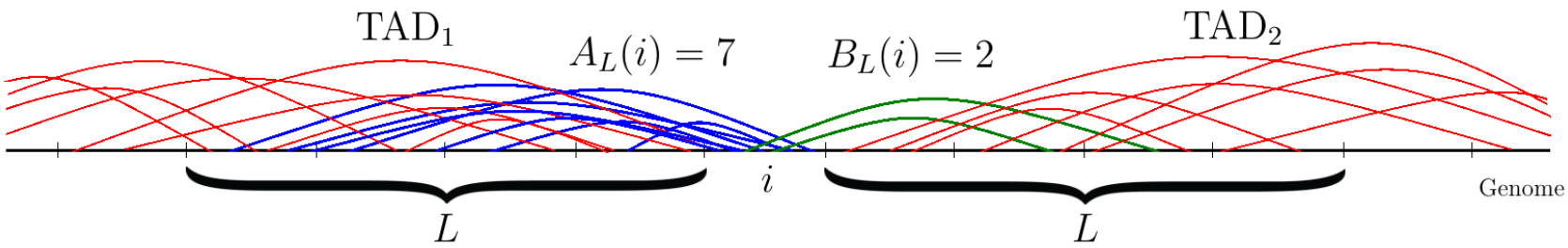


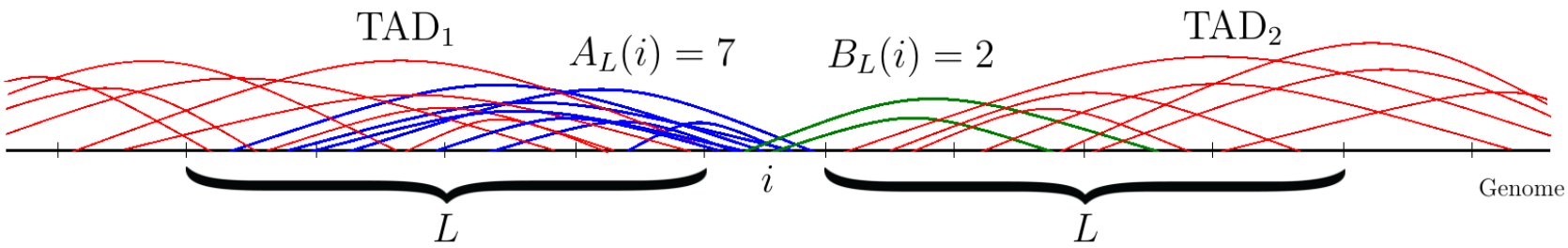
- Let's partition each chromosome into r bp bins, where r is a contact matrix resolution.
- Contacts within the chromosome can then be visualized like this. Each arc denotes a pair of reads.
- Then $A_L(i)$ is a number of read pairs that map from the bin i to the upstream L bp.
- And $B_L(i)$ is a number of read pairs that map from the bin i to the downstream L bp.

Directionality index



- At the end of a TAD we expect a bias in contact frequency towards upstream regions.

Directionality index



- At the end of a TAD we expect a bias in contact frequency towards upstream regions.
- And vice versa: at the beginning of a TAD we expect a bias in contact frequency towards downstream regions.

Directionality index

- We can use this bias for TAD calling. Consider some bin i and its L bp vicinity. Let $A \equiv A_L(i)$, $B \equiv B_L(i)$, $D \equiv D_L(i)$, and $E \equiv E_L(i)$. Then, let's define **directionality index** (Dixon et al., 2012)

$$DI = \frac{B - A}{|B - A|}\left(\frac{(A - E)^2}{E} + \frac{(B - E)^2}{E}\right),$$

where $E \equiv E_L(i) = \frac{A_L(i)+B_L(i)}{2}$ is an expected number of reads (without the upstream or downstream contact frequency bias).

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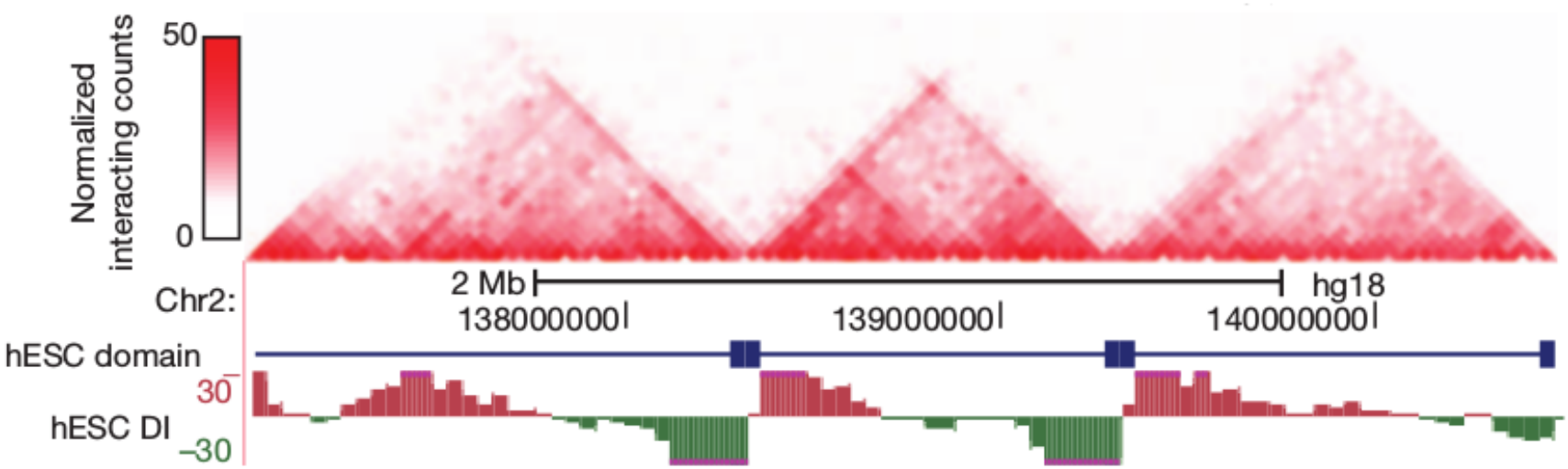
- At the end of a TAD DI should have a local minimum, and immediately at the beginning of the next TAD DI should have a local maximum.

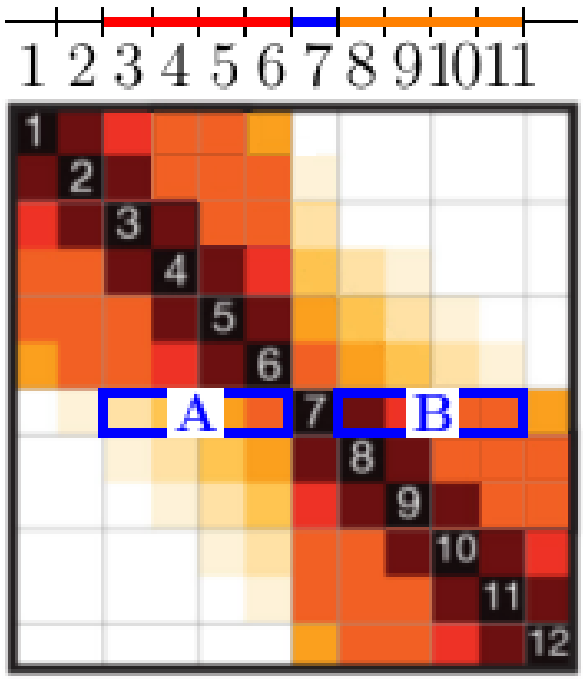
Directionality index

An illustration of this idea from Dixon et al., 2012 (Hi-C data for hESC – human embryonic stem cell line, some region of chr2):



Frame Title

DI calculation from a contact matrix (fig. is based on Crane et al., 2015):



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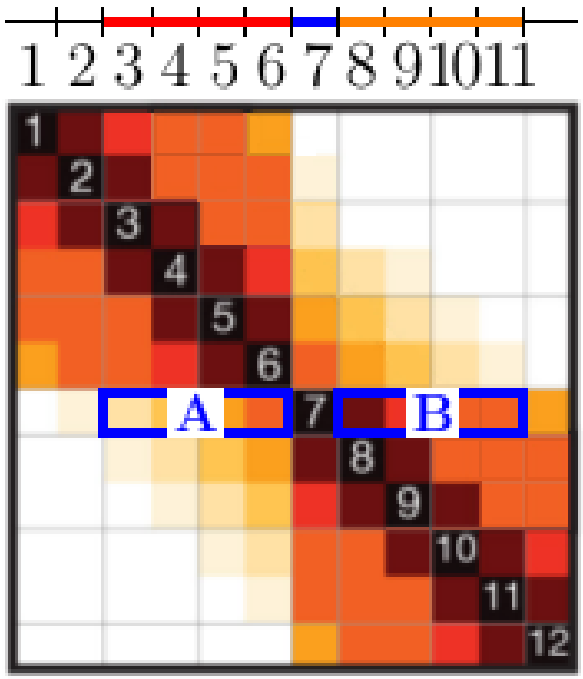
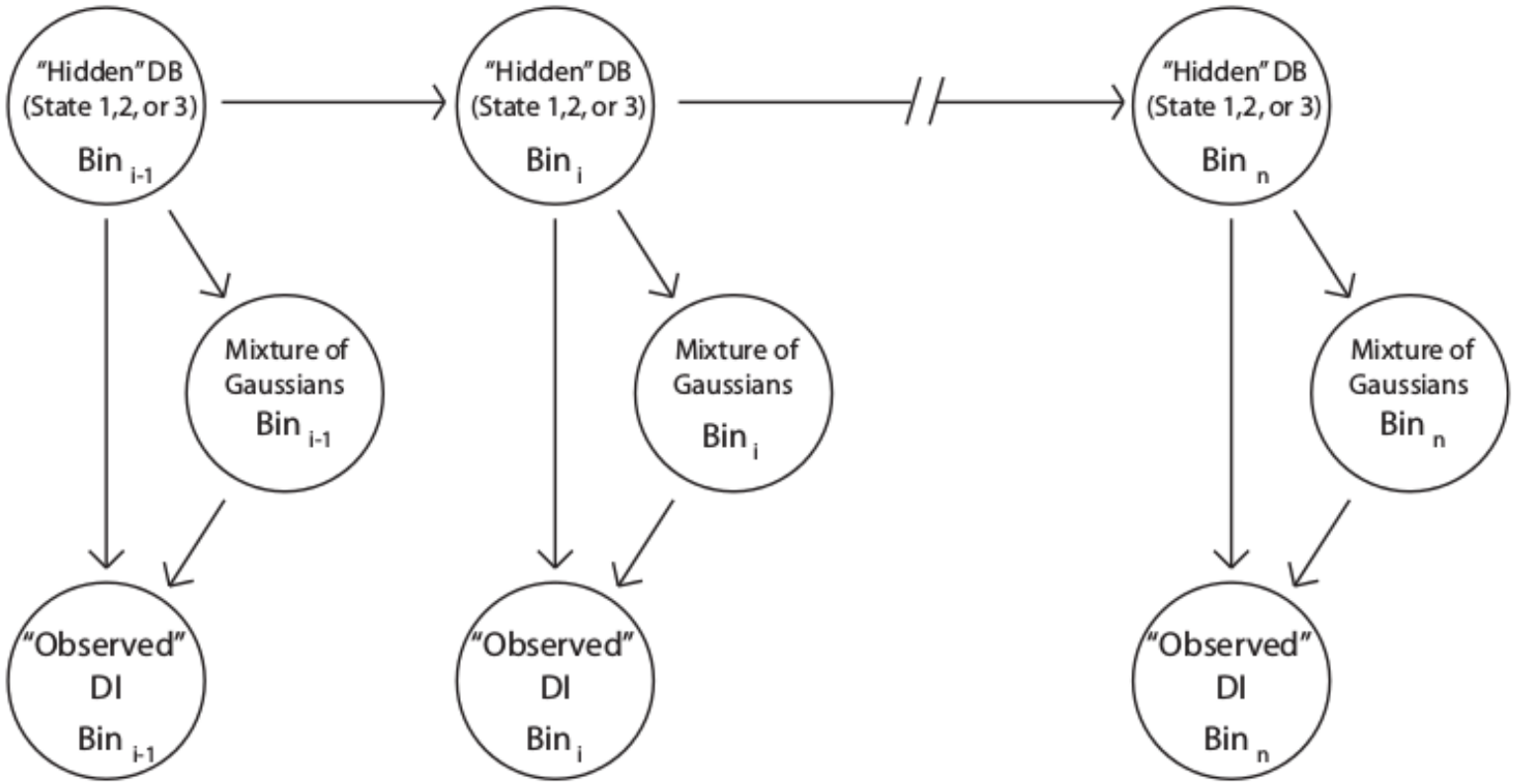


$$\mathrm{DI} = \frac{\sum_{\mathbf{B}} - \sum_{\mathbf{A}}}{|\sum_{\mathbf{B}} - \sum_{\mathbf{A}}|}\left(\frac{(\sum_{\mathbf{A}} - E)^2}{E} + \frac{(\sum_{\mathbf{B}} - E)^2}{E}\right),$$

where $E = \frac{\sum_{\mathbf{A}} + \sum_{\mathbf{B}}}{2}$, $\sum_{\mathbf{A}}$ and $\sum_{\mathbf{B}}$ are sums of elements in contact submatrices **A** and **B**, respectively.

Directionality index

Now we can define a Hidden Markov Model (HMM) for TAD calling with DI (Dixon et al., 2012):



"Upstream Bias" - State 1
"Downstream Bias" - State 2
No Bias - State 3

Directionality index

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- Forward-backward algorithm was used to estimate posterior marginals, i. e., $\Pr(Q_t = q \mid D_1 = d_1, D_2 = d_2, \ldots, D_n = d_n)$, where q is a hidden state, $t \in \{1, \ldots, n\}$, $d_1, d_2, \ldots, d_n$ are emission values.

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- For each chromosome the authors tried to use $1 - 20$ mixtures of Gaussians and chose one set with the best goodness of fit using the AIC criterion: $\mathrm{AIC} = 2k - 2\ln(L)$, where k is the number of parameters in the model and L is the maximum likelihood estimate.

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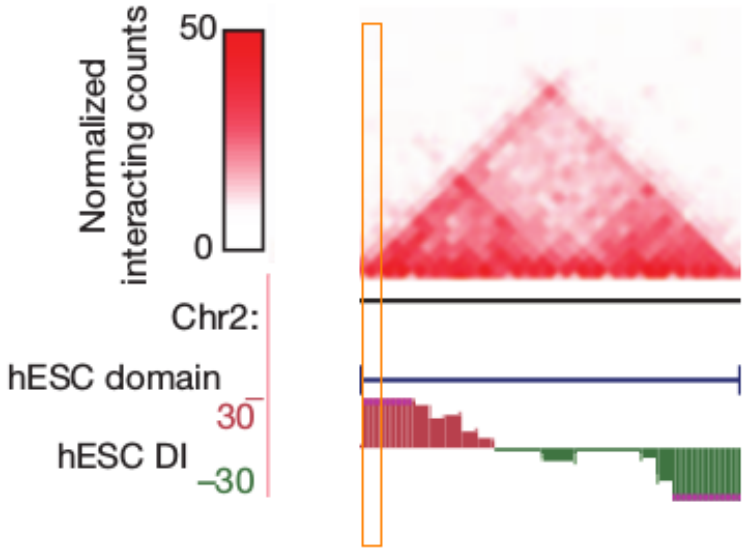
- **TAD calling:**
  - TAD begins at the beginning of the first `DB` state in a series of `DB` states.



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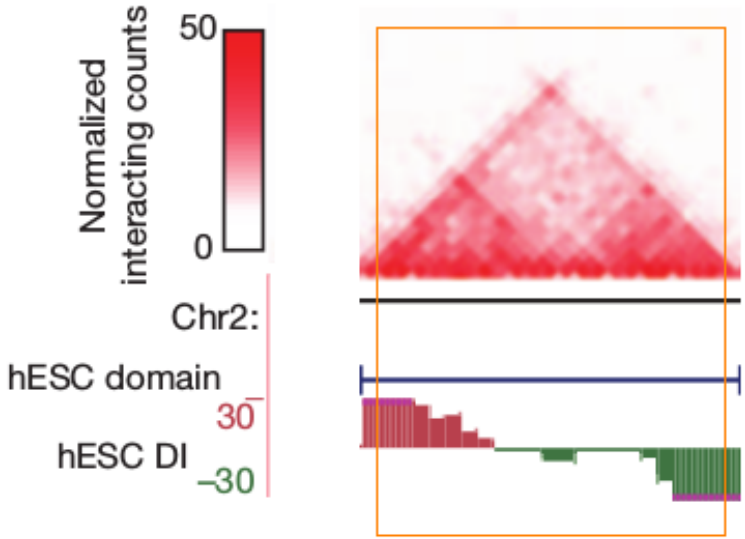

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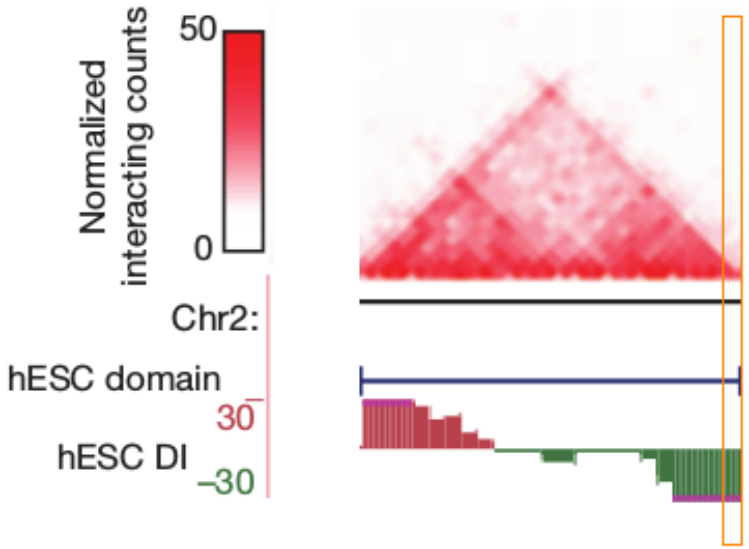

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- **TAD borders:** a region between TADs is called **topological boundary** if its length is less than 400 kbp, otherwise it is called **unrecognized chromatin**.
- Topological boundaries in mouse ESC were found to be quite small, 76.33 % of them being less than 50 kbp.

Directionality index

The main biological results in Dixon et al., 2012 are as follows:

- TADs were called in mouse and human ESC, as well as in some terminally differentiated cell types. E. g., about 91 % of the mouse ESC is occupied by TADs with median size around 880 kbp.

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Directionality index

Although Dixon et al., 2012 didn't publish their scripts (they used MATLAB) and detailed description of the HMM, directionality index (DI) became a popular metric for TAD calling.

Directionality index

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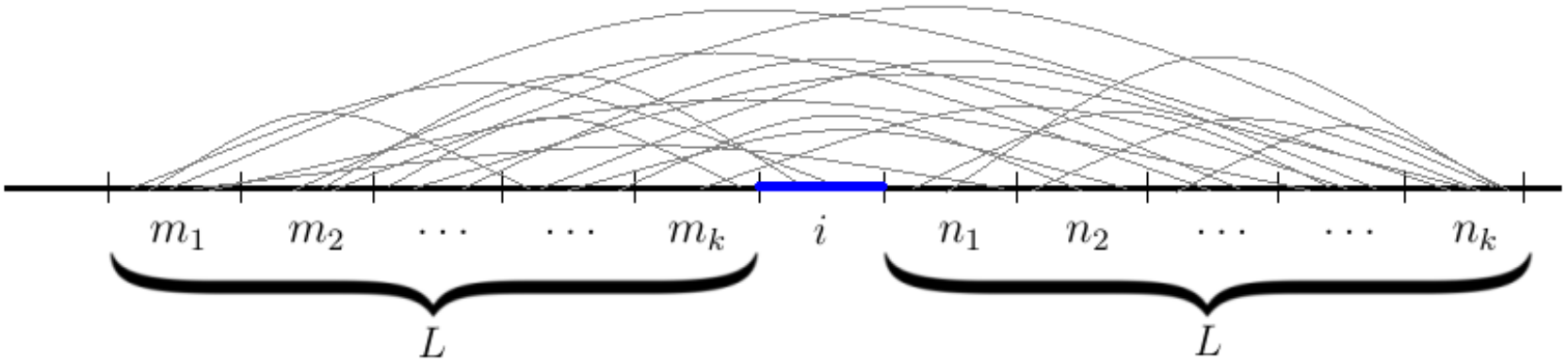
- Dileep et al., 2015 calculated DI in six regions at several time points in the G1-phase of mouse mammary epithelial cell line (C127) watching a switch from a negligible to strong directionality bias that suggested formation of TADs.

Insulation score



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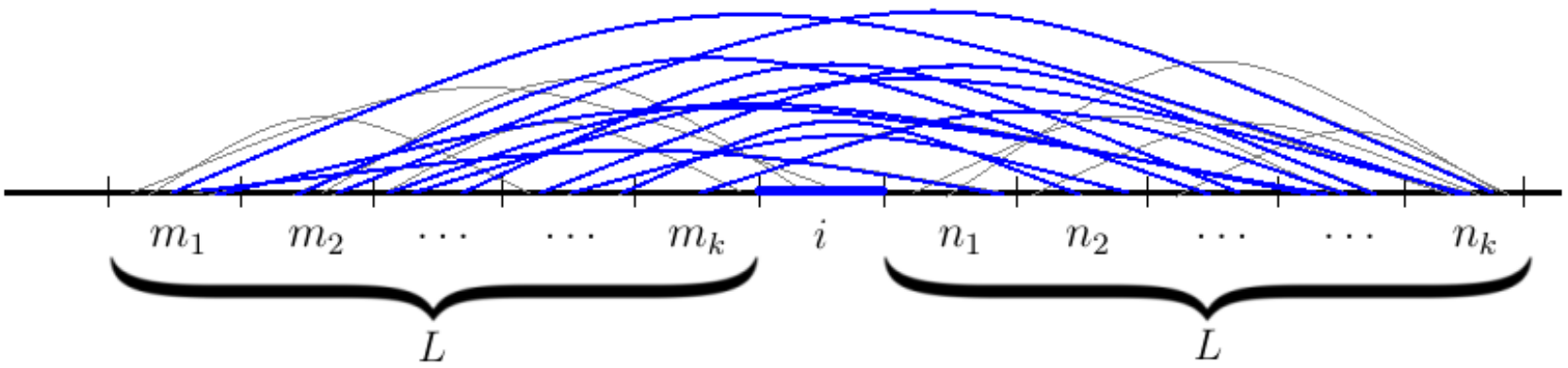

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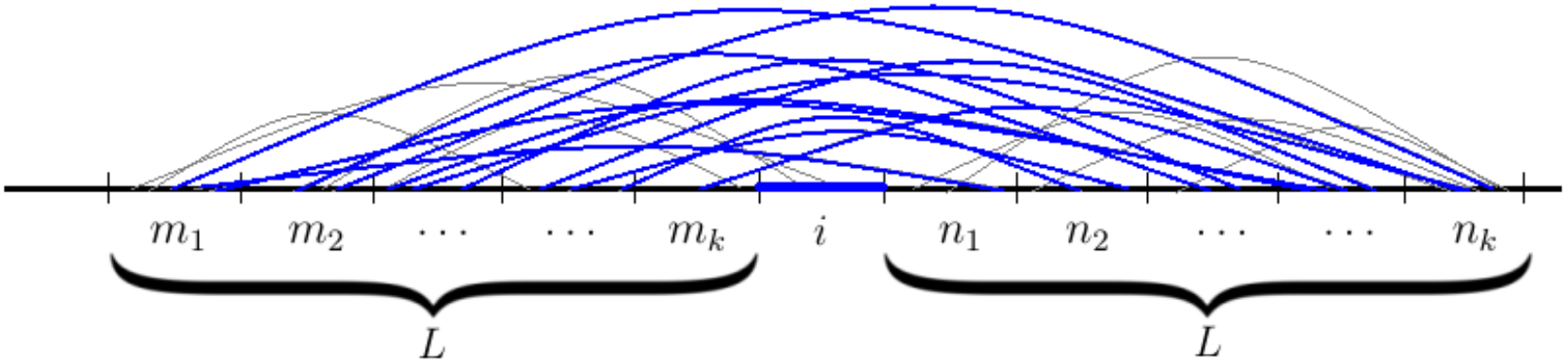

Insulation score (IS) is defined for a bin as an average number of interactions that occur across this bin in some vicinity of the bin (Crane et al., 2015):

$$\text{IS} = \frac{1}{k^2} \sum_{m \in M,\, n \in N} C(m, n),$$

where $N = \{n_1, n_2, \ldots, n_k\}$, $M = \{m_1, m_2, \ldots, m_k\}$, $C(m, n)$ is a number of interactions between bin m and bin n.

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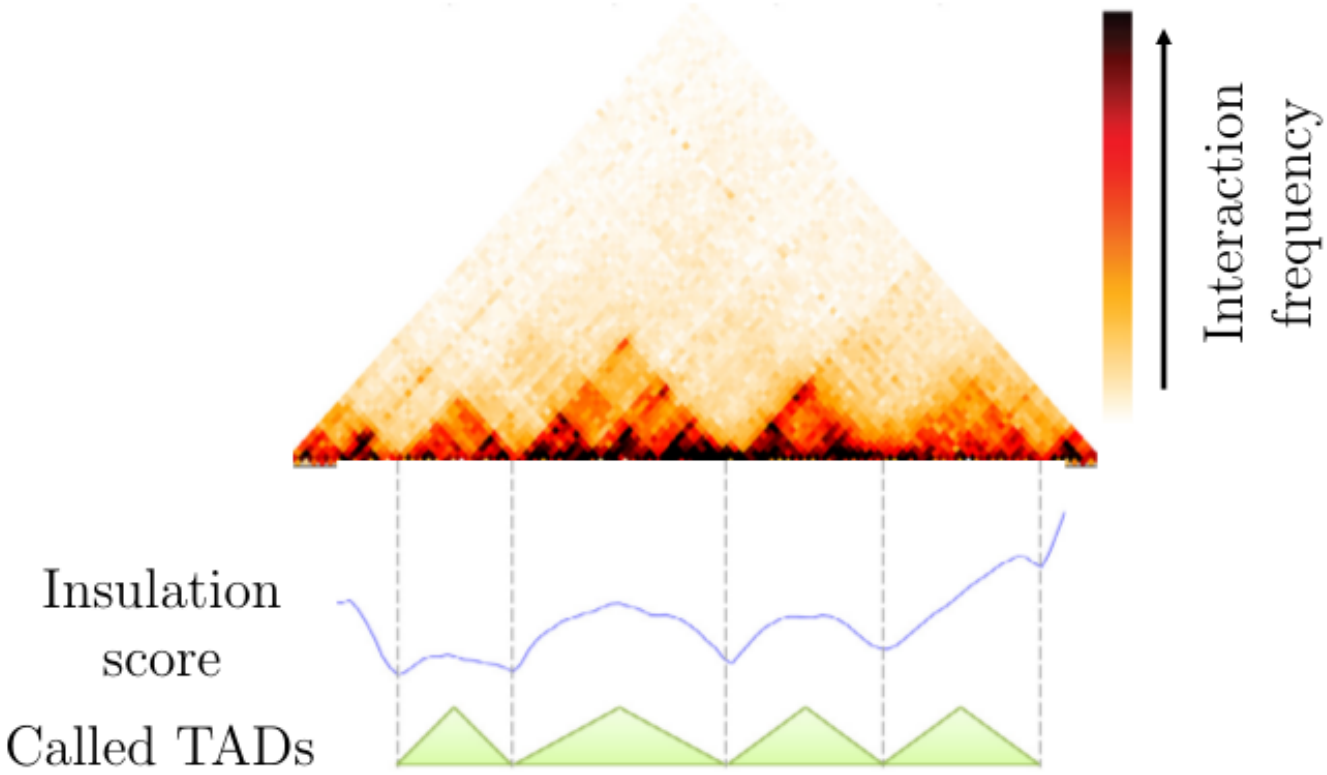

Lajoie et al., 2015, adapted

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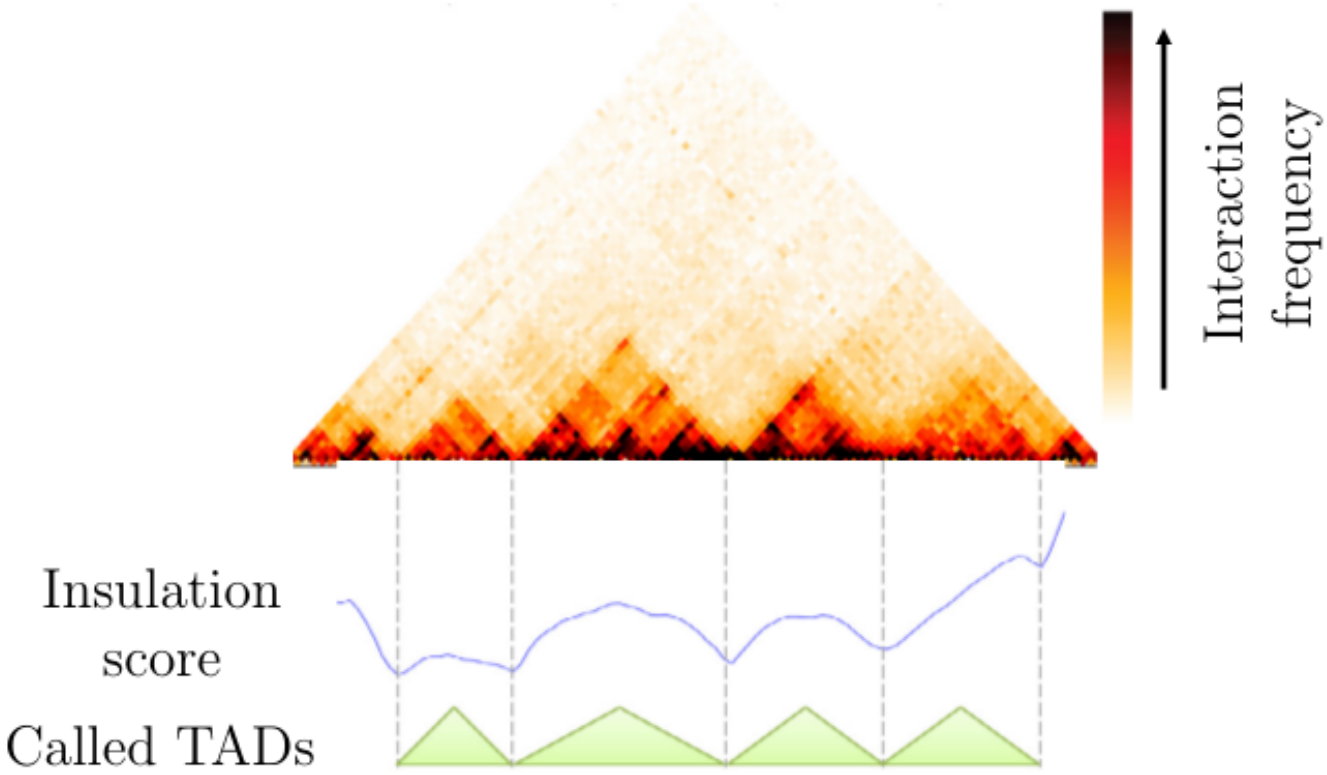

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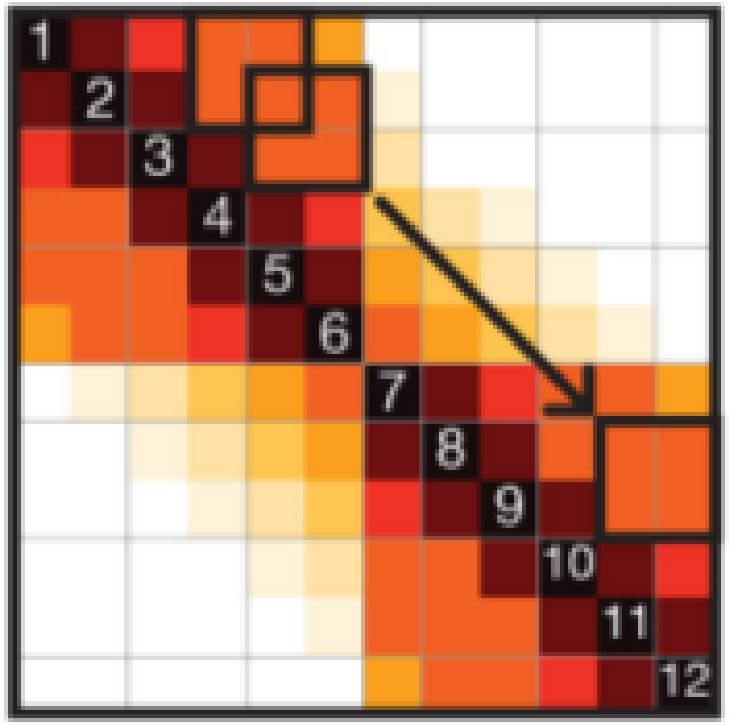
- We expect that IS has local minimums at TAD borders.
- IS plot is often called **insulation profile**.

Insulation score

IS can be calculated using a square window sliding along the diagonal of a contact matrix: average number of interactions in this window is the insulation score value (Crane et al., 2015, adapted):



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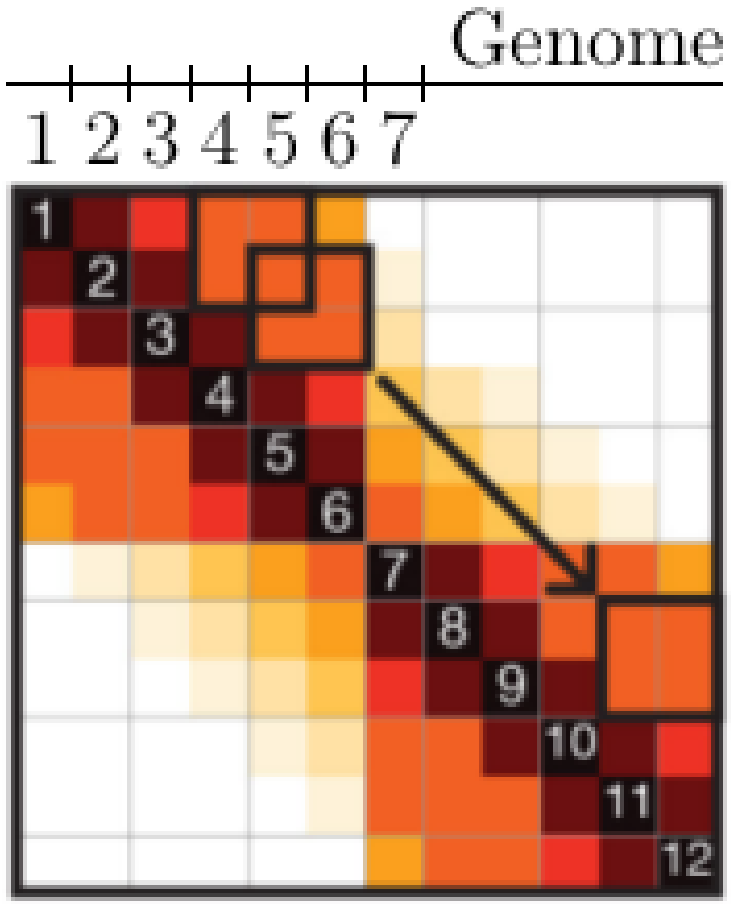

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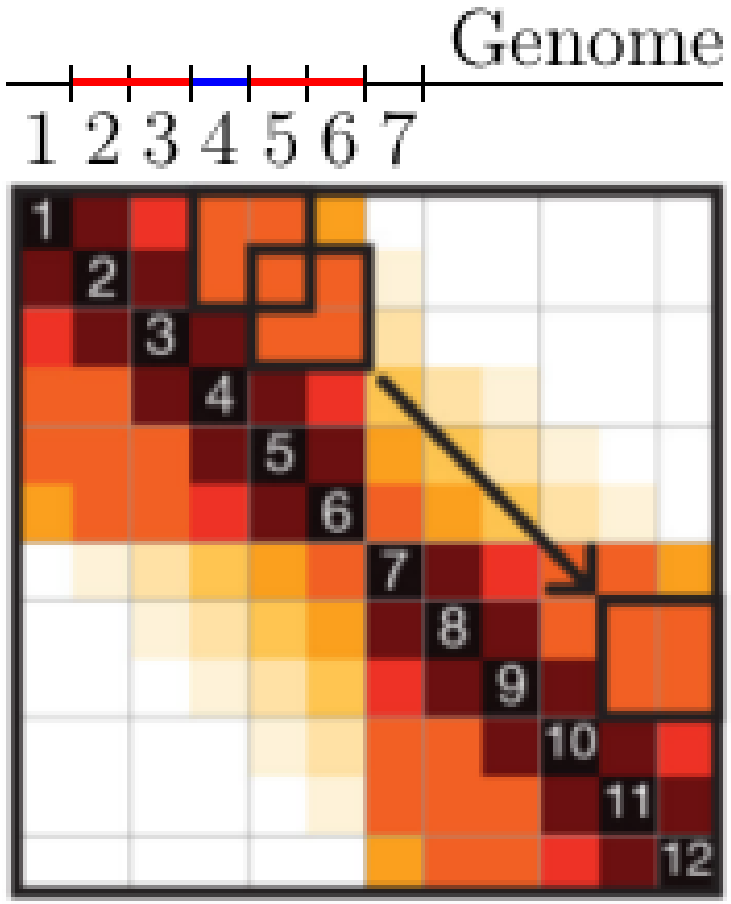

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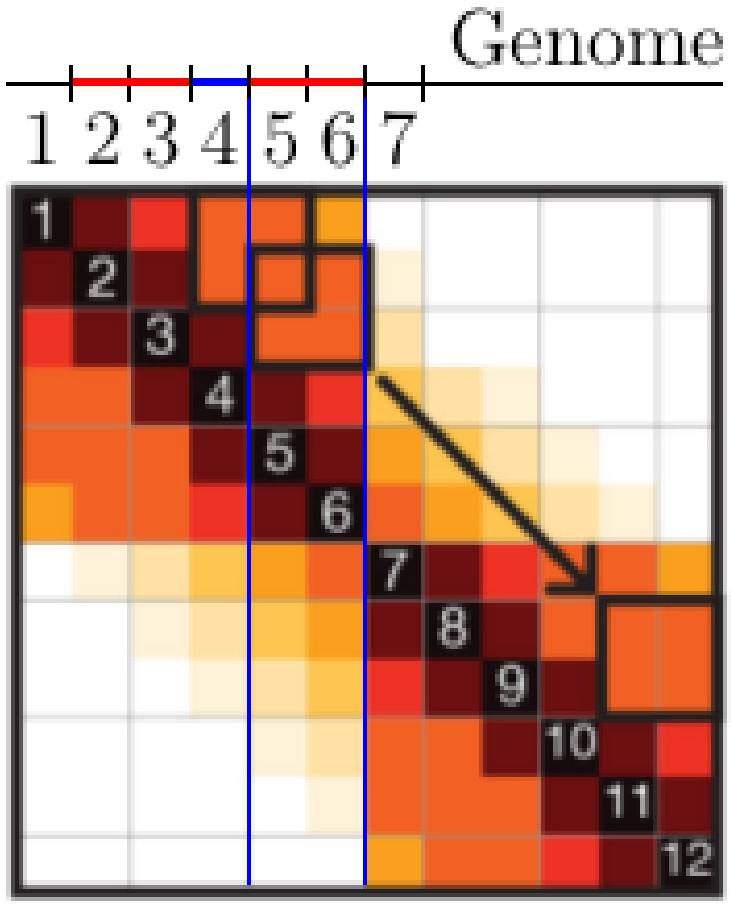

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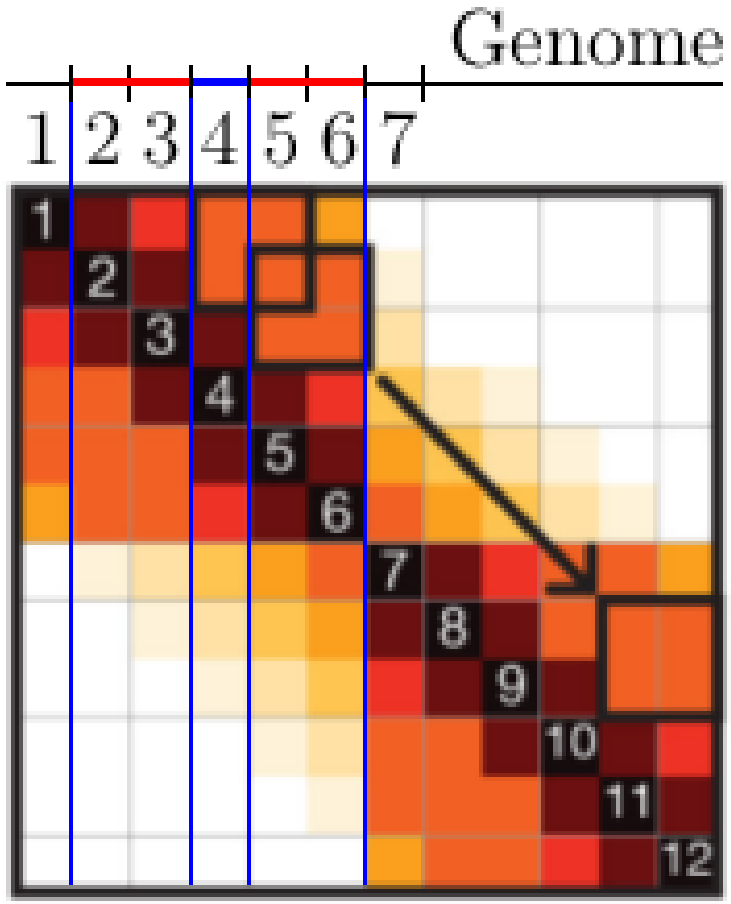

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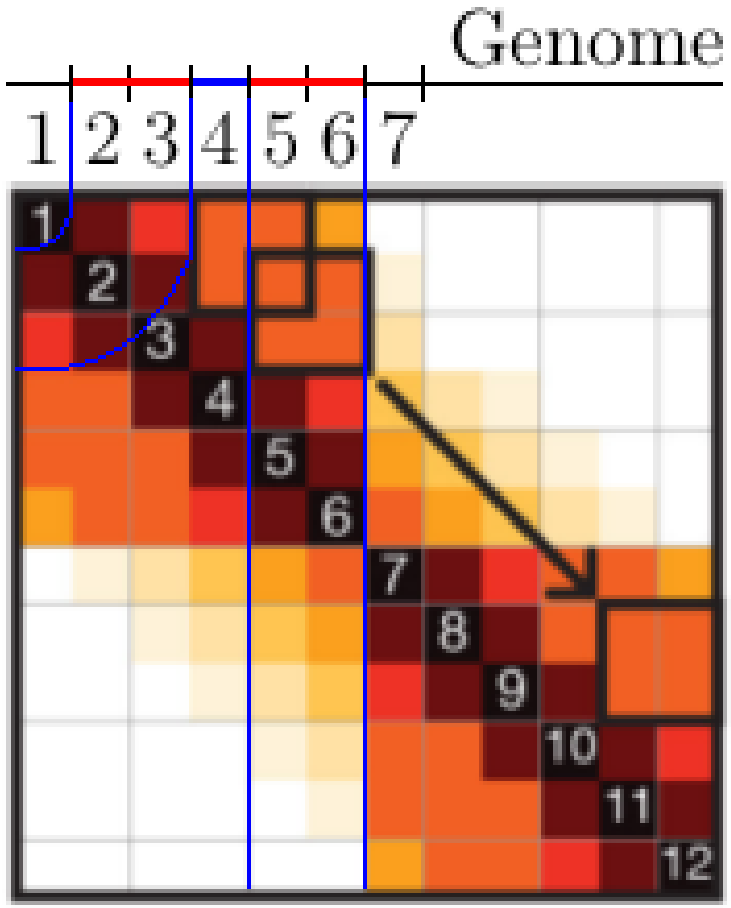

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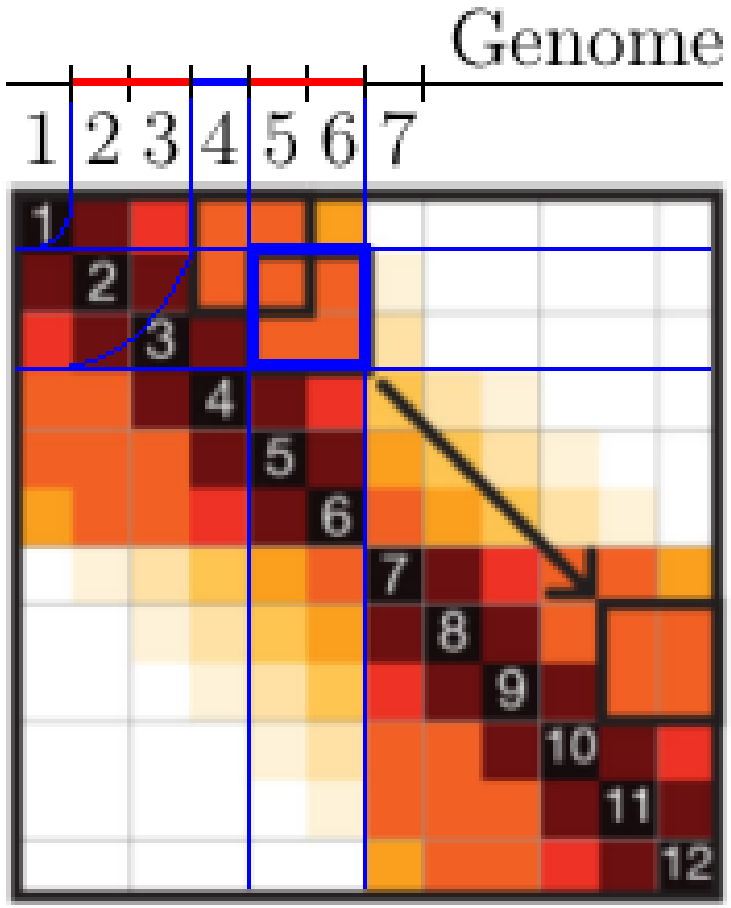

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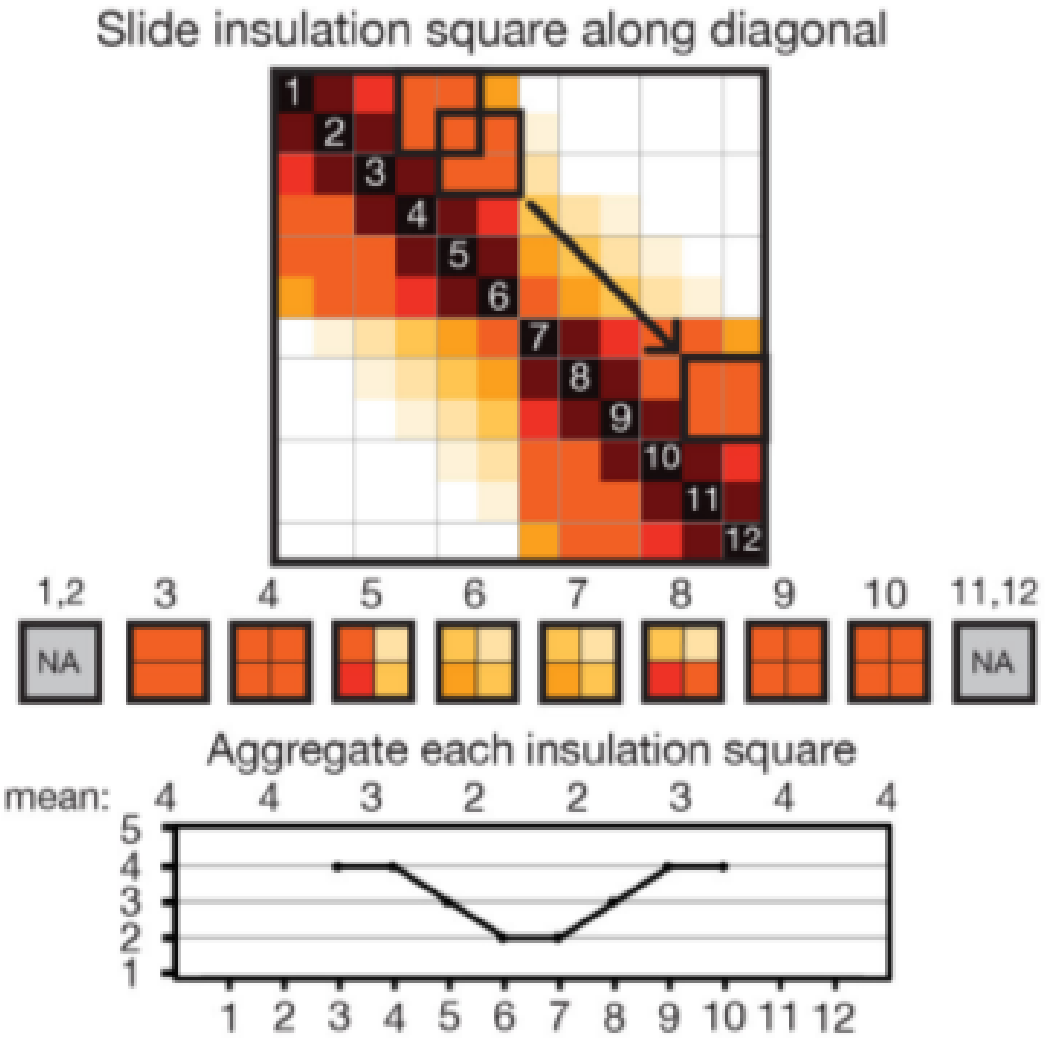
IS calculation scheme (Crane et al., 2015):



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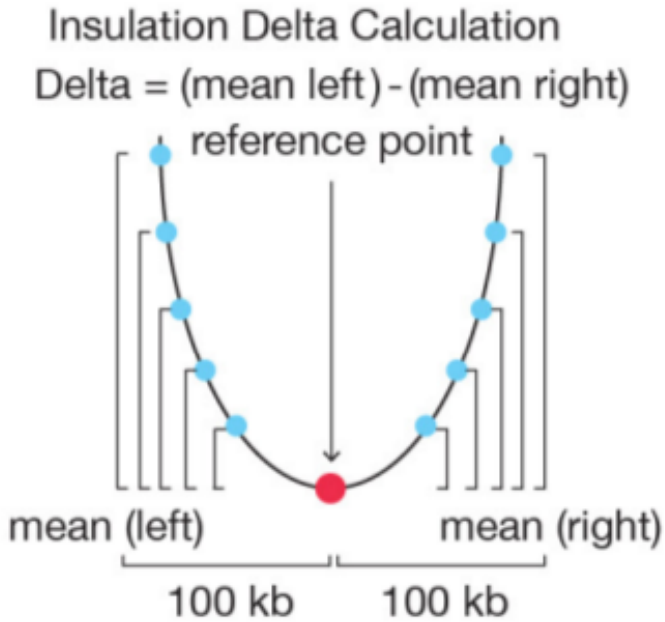

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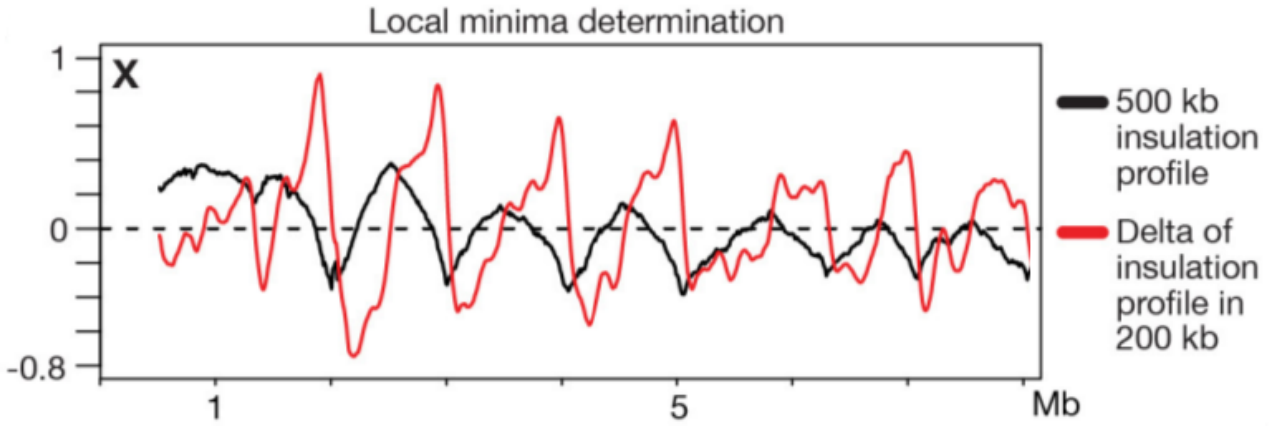

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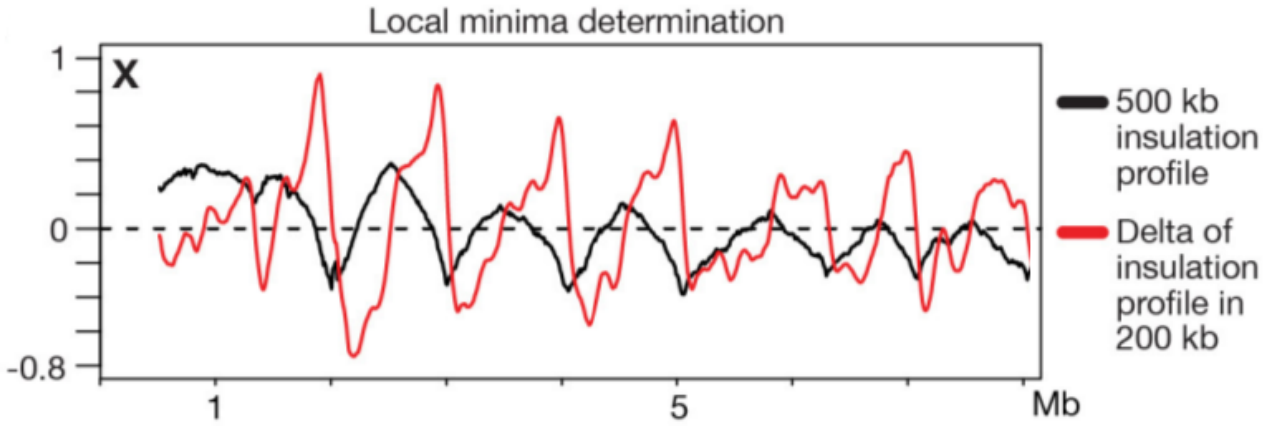

- TAD border is called at bin i if $\Delta_i = 0$, the nearest Δ local max (Δ_{max}) is to the left of bin i, the nearest Δ local min (Δ_{min}) is to the right, and $S_i \equiv \Delta_{\mathrm{max}} - \Delta_{\mathrm{min}} > 0.1$. S_i is called **border (boundary) strength**. TAD is called between two borders.

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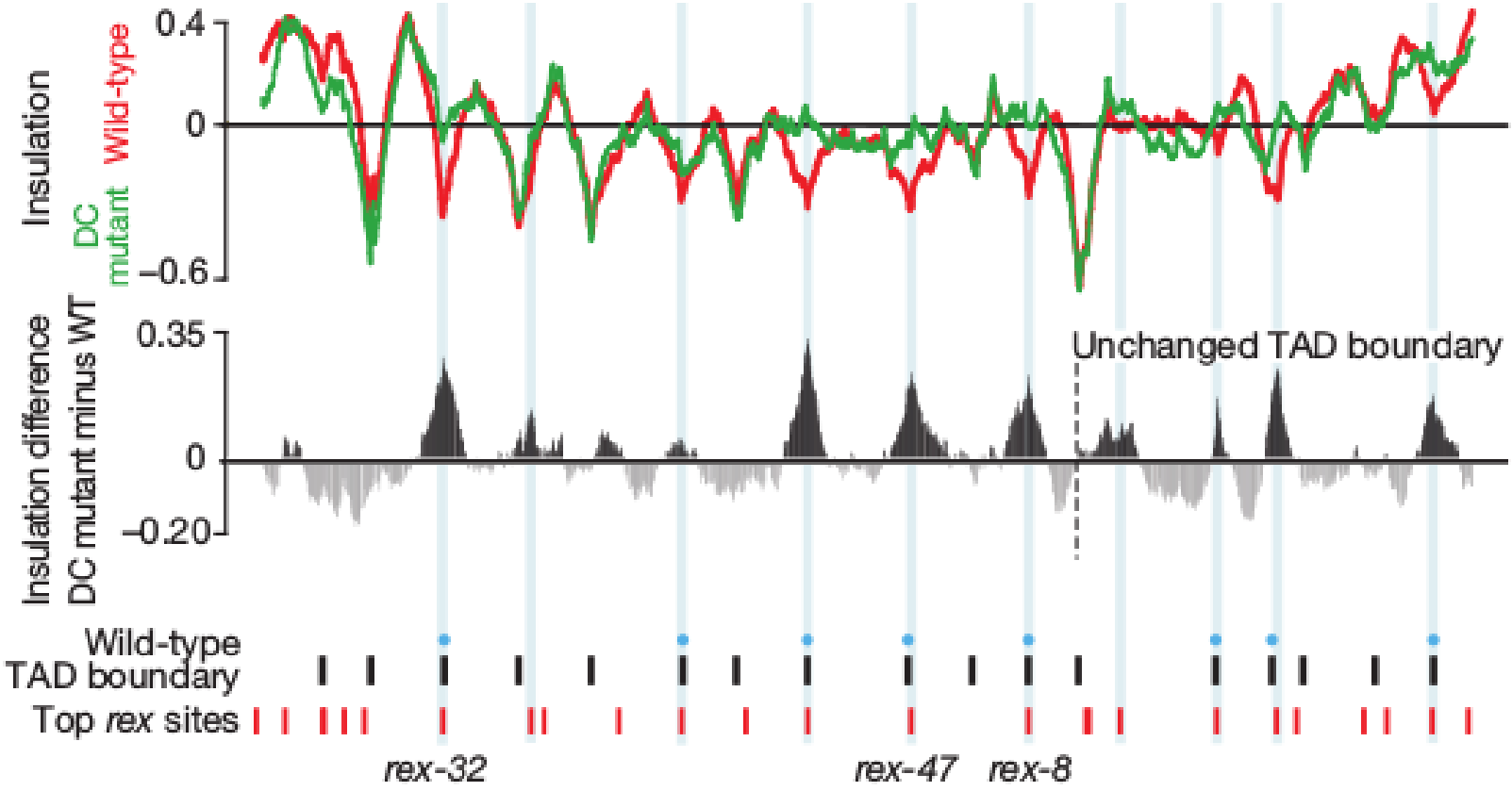

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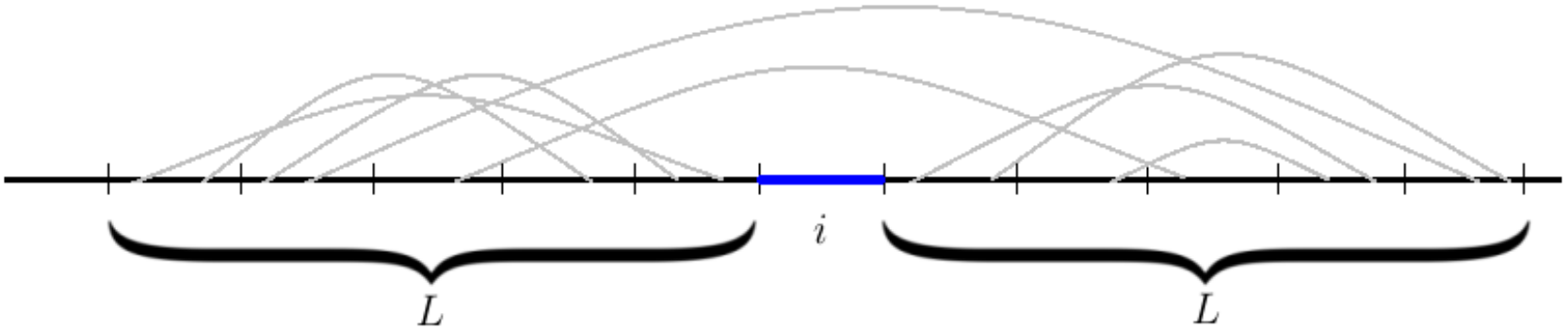
- They called TAD borders with IS to see how they change in *C. elegans* X chromosome due to dosage compensation complex (DCC) depletion.
- Barutcu et al., 2015 called TADs with IS to see differences in higher order chromatin structure between MCF-10A mammary epithelial and MCF-7 breast cancer cell lines.

Contrast index



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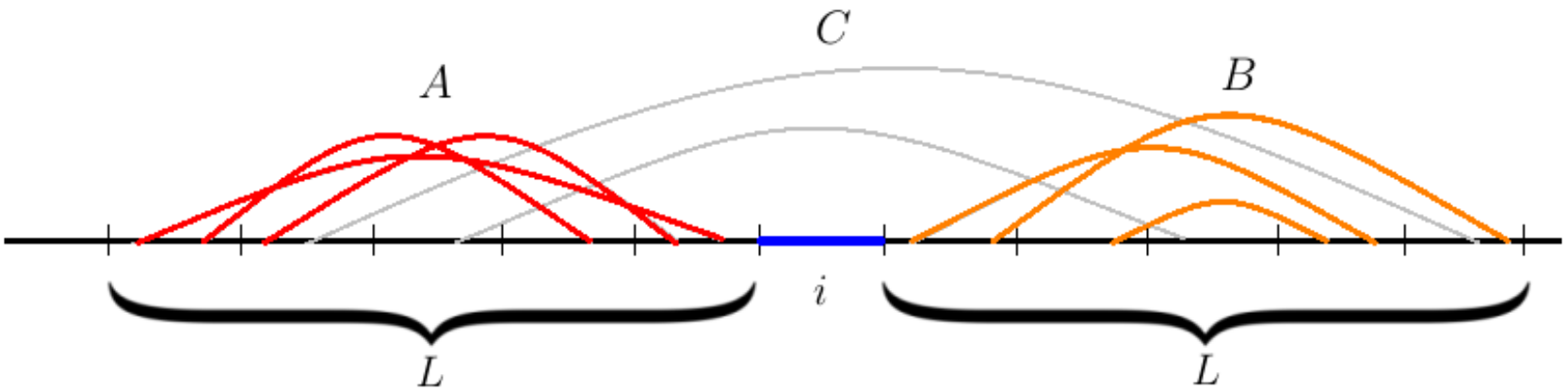

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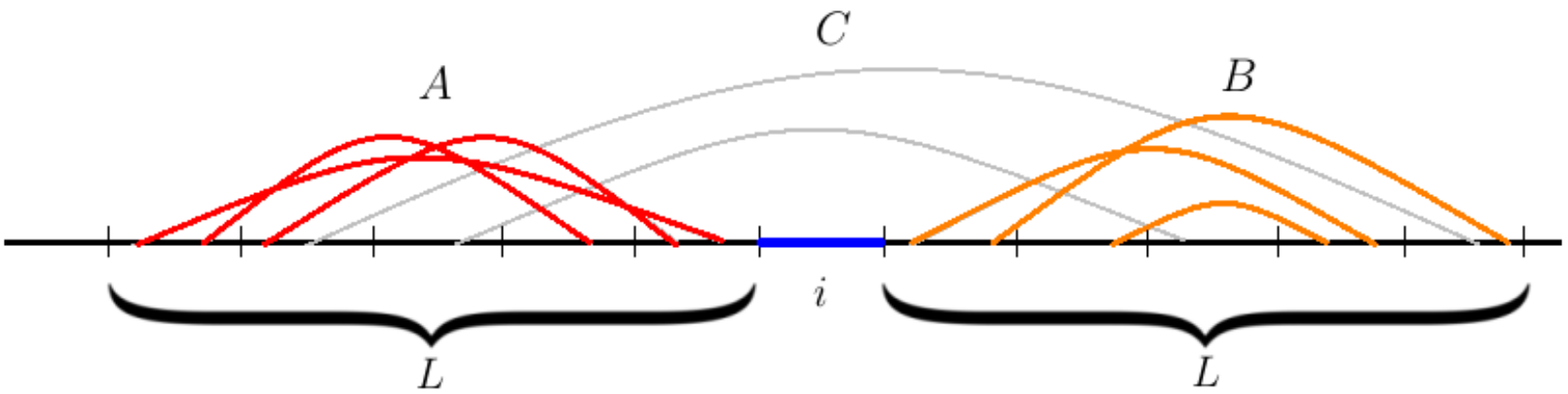


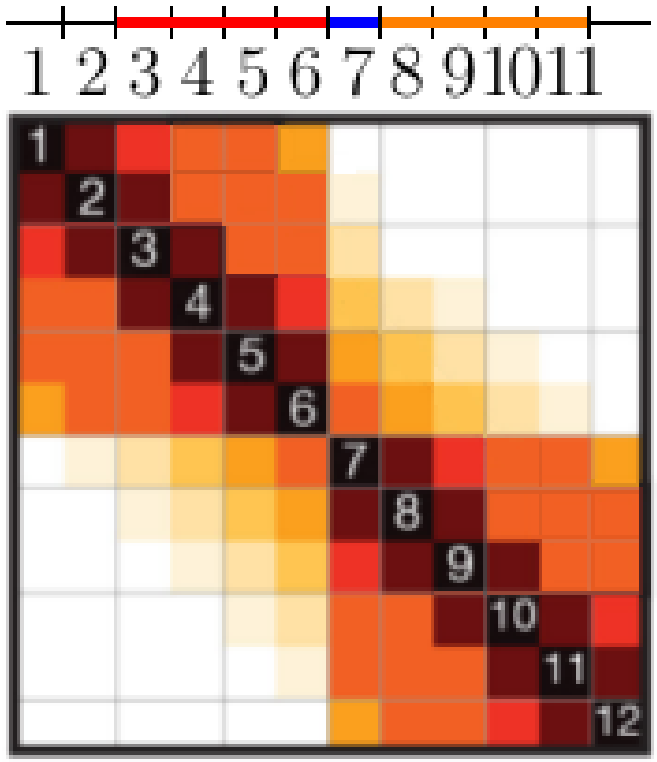
Contrast index is defined as follows (Van Bortle et al., 2014, Alekseyenko et al., 2015):

$$\mathrm{CI} = \frac{A + B}{C},$$

where A is a total number of interactions to the left of bin i in L-vicinity, B is a total number of interactions to the right of bin i in L-vicinity, and C is a number of interactions that occur over bin i from the left L-vicinity to the right.

Contrast index

CI calculation using a contact matrix (fig. is based on Crane et al., 2015):



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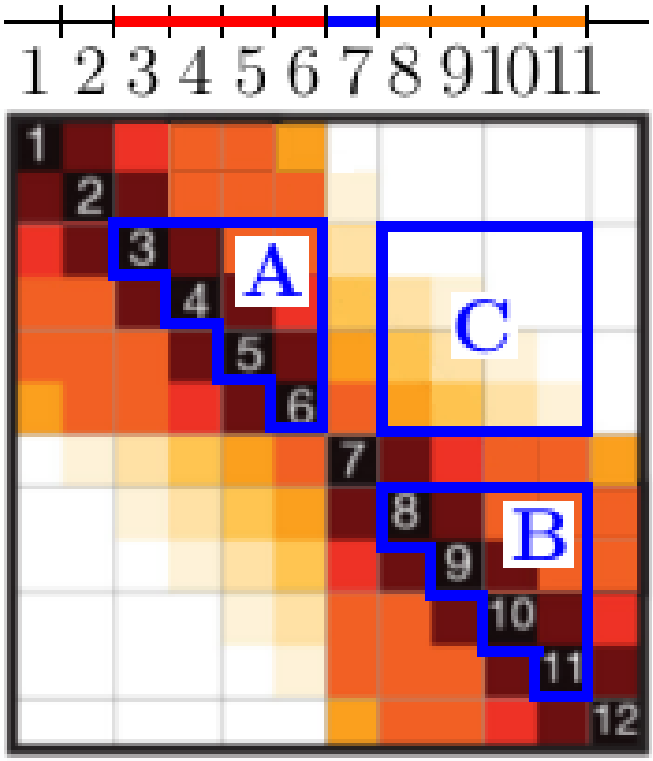

$$\mathrm{CI} = \frac{\sum_{\mathbf{A}} + \sum_{\mathbf{B}}}{\sum_{\mathbf{C}}},$$

where $\sum_{\mathbf{A}}$, $\sum_{\mathbf{B}}$, $\sum_{\mathbf{C}}$ are sums of elements in **A**, **B**, and **C** contact submatrices, respectively.

Contrast index

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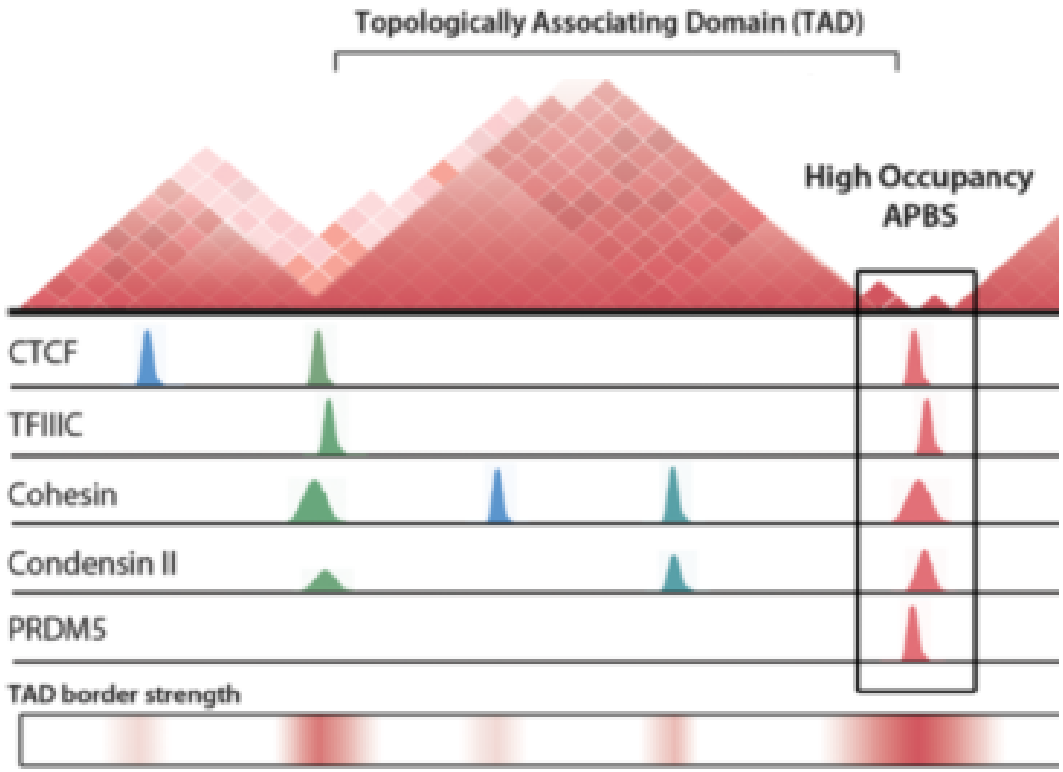

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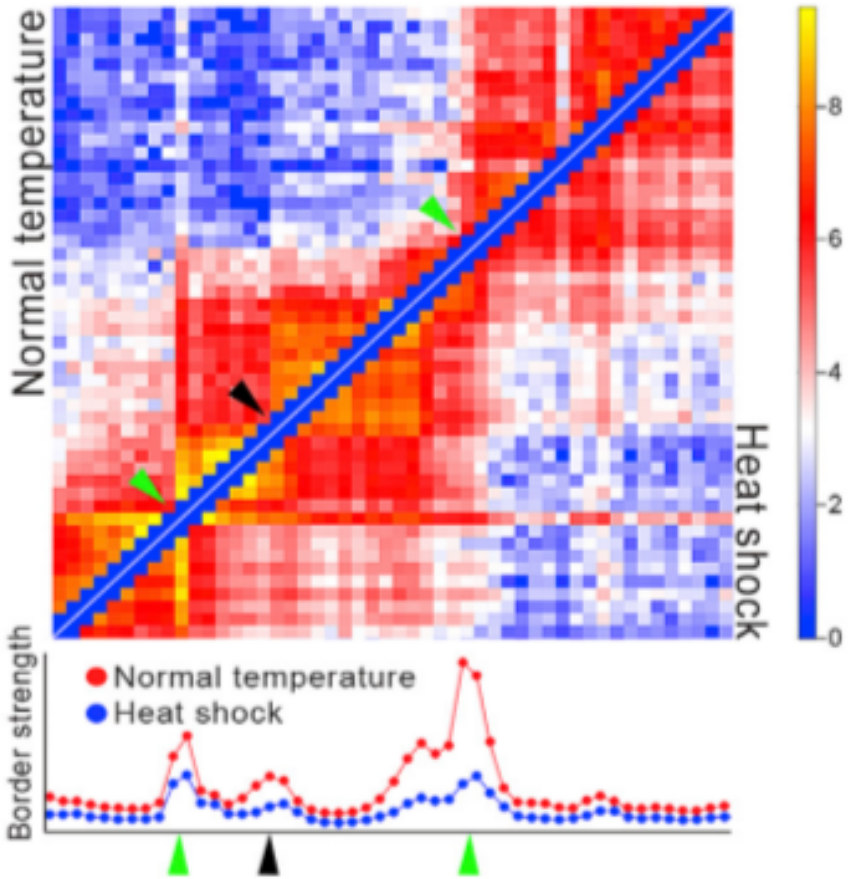
- CI was used for TAD calling and TAD border strength assessment in several papers. E. g.:
  - Li et al., 2015 studied TAD border strength decline in *Drosophila* cells after heat-shock:



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- **In Part 2** I'll consider *some* of the following much more complicated methods and tools for TAD calling: Sexton et al., 2012; Hou et al., 2012; Armatus, 2014; HiCseg, 2014; Arrowhead algorithm, 2014; TADtree, 2015; TADbit.

Outline

Chromatin conformation overviews

- Nguyen H. G. and Bosco G. 2015. Gene positioning effects on expression in Eukaryotes. *Annual Review of Genetics* 49: 627–646.
- Gibcus J. H. and Dekker J. 2013. The hierarchy of the 3D genome. *Molecular Cell* 49(5): 773–782.
- Dekker J. and Heard E. 2015. Structural and functional diversity of topologically associating domains. *FEBS Letters* 589(20, Part A): 2877–2884.

Self-interacting chromatin domains in various species

- **Chromatin interaction domains (CIDs) in bacterium *Caulobacter crescentus*:** Le T. B. et al. 2013. High-resolution mapping of the spatial organization of a bacterial chromosome *Science* 342(6159): 731–734.
- **Chromatin globules in *S. pombe*** Mizuguchi T. et al. 2014. Cohesin-dependent globules and heterochromatin shape 3D genome architecture in S. pombe . *Nature* 516(7531): 432–435.
- **Physical domains in *Drosophila:*** Sexton T. et al. 2012. Three-dimensional folding and functional organization principles of the Drosophila Genome. *Cell* 148(3): 458–472.
- **TADs in *C. elegans*** Crane E. et al. 2015. Condensin-driven remodeling of X-chromosome topology during dosage compensation. *Nature* 523(7559): 240–244.
- **TADs in human and mouse:** Dixon J. R. et al. 2012. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485(7398): 376–380.

Chromatin conformation capture methods:

- **Overview:** de Wit E. and de Laat W. 2012. A decade of 3C technologies: insights into nuclear organization. *Genes & Development* 26(1): 11–24.
- **Hi-C:** Lieberman-Aiden E. et al. 2009. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* 326(5950): 289–293.
- **Some Hi-C derivatives:**
  In-situ Hi-C: Rao S. S. et al. 2014. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 159(7): 1665–1680.
  Capture Hi-C: Mifsud B. et al. 2015. Mapping long-range promoter contacts in human cells with high-resolution capture Hi-C. *Nature Genetics* 47(6): 598–606.

Hi-C data processing and analysis

Overviews:

- Lajoie B. R. et al. 2015. The Hitchhiker's guide to Hi-C analysis: practical guidelines. *Methods* 72: 65 – 75.
- Ay F. and Noble W. S. 2015. Analysis methods for studying the 3D architecture of the genome. *Genome Biology* 16:183.

Hi-C data correction:

- Imakaev M. et al. 2012. Iterative correction of Hi-C data reveals hallmarks of chromosome organization. *Nature Methods* 9(10): 999–1003.
- Yaffe E. and Tanay A. 2011. Probabilistic modeling of Hi-C contact maps eliminates systematic biases to characterize global chromosomal architecture. *Nature Genetics* 43(11): 1059–1065.

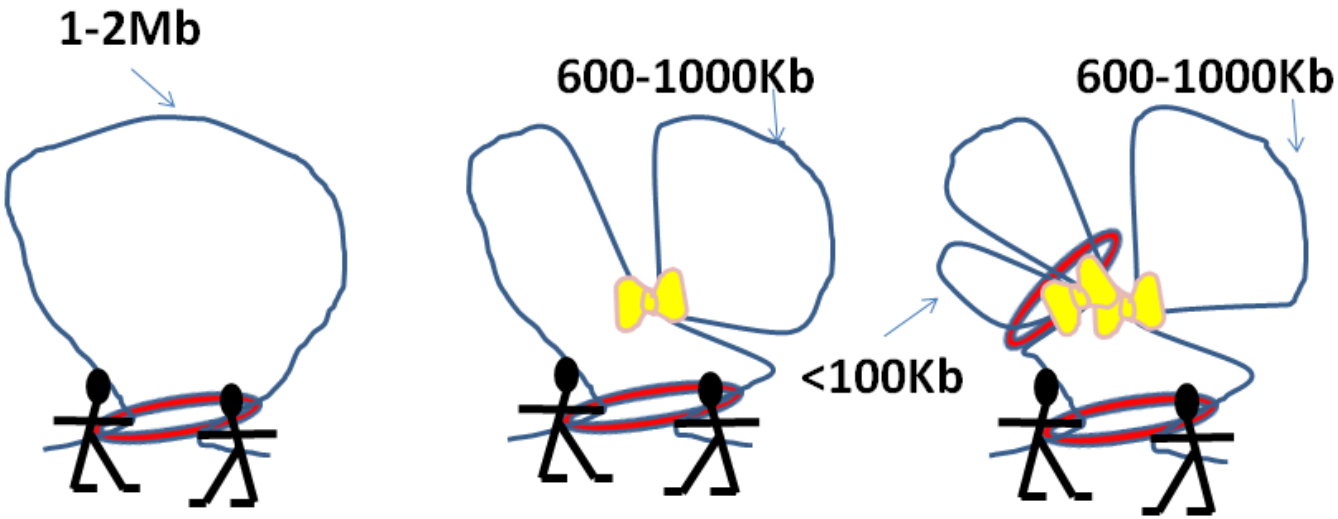
TAD calling methods

Covered in this overview:

- **Directionality index:** Dixon J. R. et al. 2012. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485(7398): 376–380.
- **Insulation score:** Crane E. et al. 2015. Condensin-driven remodeling of X-chromosome topology during dosage compensation. *Nature* 523(7559): 240–244.
- **Contrast index:** Van Bortle K. et al. 2014. Insulator function and topological domain border strength scale with architectural protein occupancy. *Genome Biology* 15(6): R82.
  Alekseyenko A. A. et al. 2015. The oncogenic BRD4-NUT chromatin regulator drives aberrant transcription within large topological domains *Genes & Development* 29(14): 1507–1523.

Additional: $\log_2$**-ratio:** Mizuguchi T. et al. 2014. Cohesin-dependent globules and heterochromatin shape 3D genome architecture in S. pombe *Nature* 516(7531): 432–435.

Thank you!



Sam Rose. Epigenetics and organisation