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Chromatin and 3C approaches

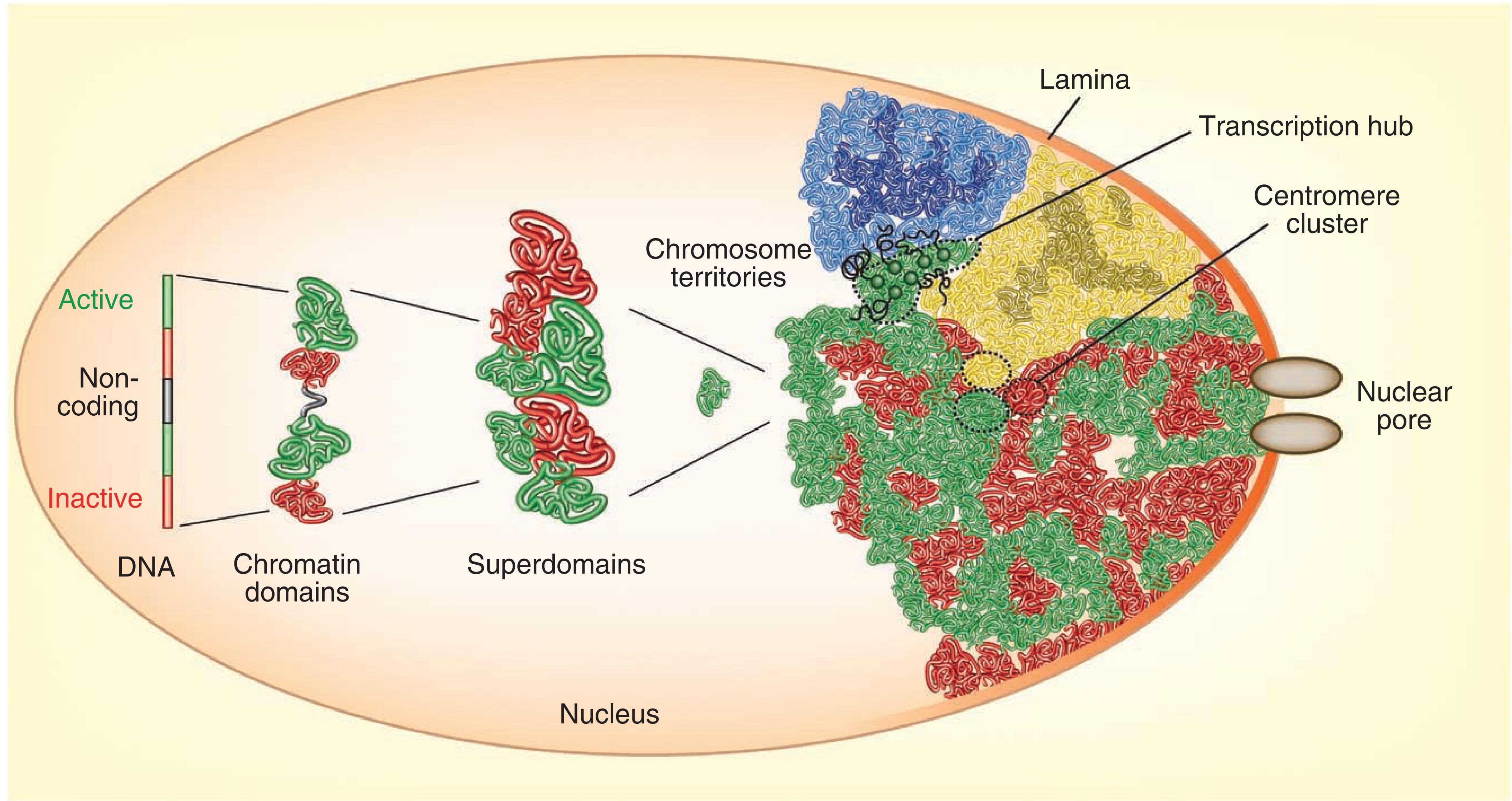
Marc A. Martí-Renom
CNAG-CRG · ICREA

<http://marciuslab.org>
<http://3DGenomes.org>
<http://cnag.crg.eu>

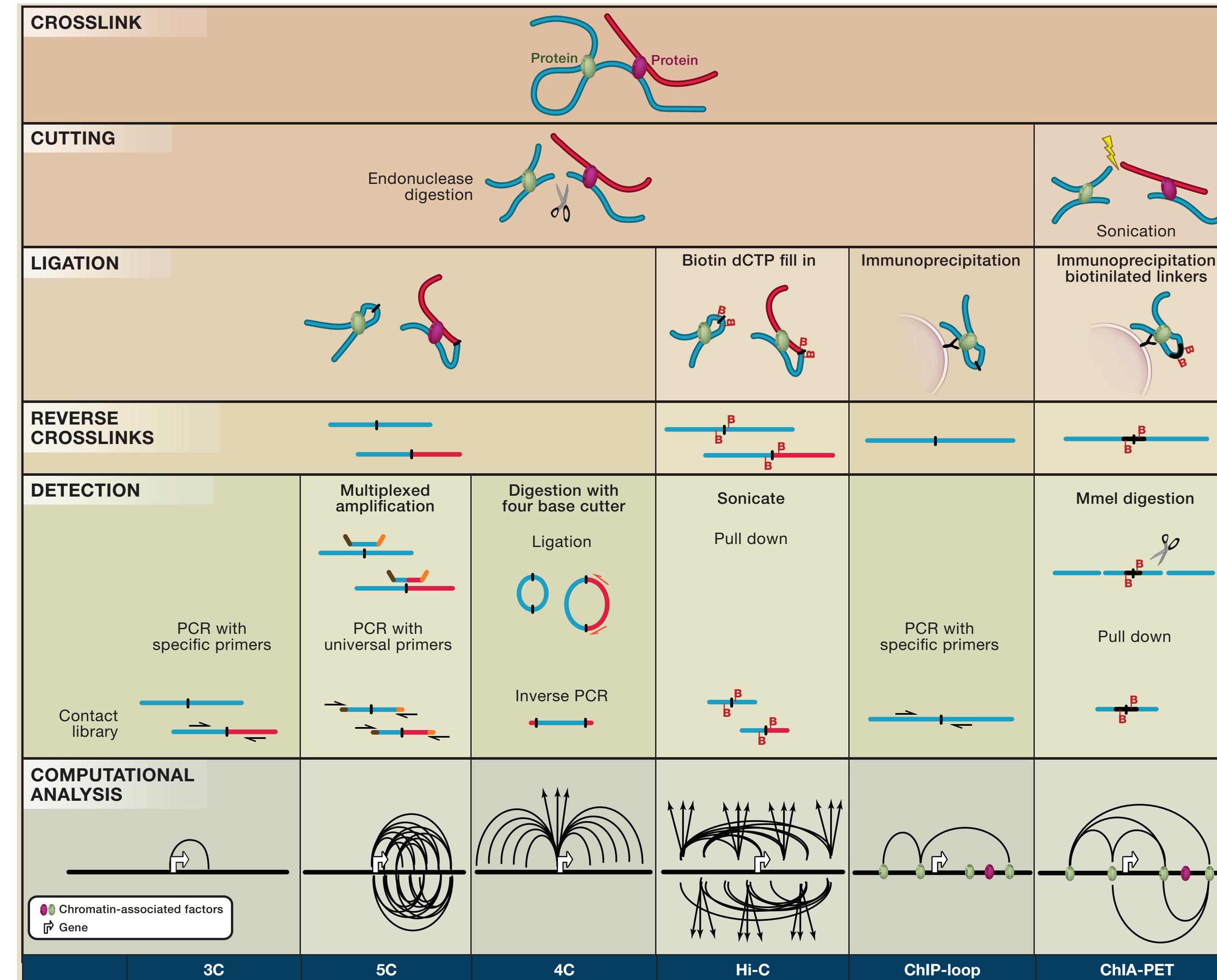
cnag CRG^R ICREA

Complex genome organization

Cavalli, G. & Misteli, T. Functional implications of genome topology. *Nat Struct Mol Biol* 20, 290–299 (2013).



Chromosome Conformation Capture



ARTICLE

doi:10.1038/nature12593

Single-cell Hi-C reveals cell-to-cell variability in chromosome structure

Takashi Nagano^{1*}, Yaniv Lubling^{2*}, Tim J. Stevens^{3*}, Stefan Schoenfelder¹, Eitan Yaffe², Wendy Dean⁴, Ernest D. Lue³, Amos Tanay² & Peter Fraser¹

LETTER

doi:10.1038/nature20158

Capturing pairwise and multi-way chromosomal conformations using chromosomal walks

Pedro Olivares-Chauvet¹, Zohar Mukamel¹, Aviezer Lifshitz¹, Omer Schwartzman¹, Noa Oded Elkayam¹, Yaniv Lubling¹, Gintaras Deikus², Robert P. Sebra³ & Amos Tanay¹

nature
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ARTICLES

<https://doi.org/10.1038/s41588-018-0161-5>

Enhancer hubs and loop collisions identified from single-allele topologies

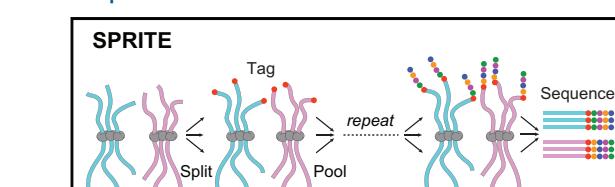
Amin Allahyar^{1,2}, Carlo Vermeulen^{3,7}, Britta A. M. Bouwman³, Peter H. L. Krijger³, Marjon J. A. M. Versteegen³, Geert Geelen³, Melissa van Kranenburg³, Mark Pieterse³, Roy Straver^{3,1}, Judith H. I. Haarhuis⁴, Kees Jalink⁵, Hans Teunissen⁶, Ivo J. Renkens¹, Wigard P. Kloosterman¹, Benjamin D. Rowland¹, Elzo de Wit⁶, Jeroen de Ridder^{3,*} and Wouter de Laat^{3*}

Resource

Cell

Higher-Order Inter-chromosomal Hubs Shape 3D Genome Organization in the Nucleus

Graphical Abstract



Authors
Sofia A. Quinodoz, Noah Ollikainen, Barbara Tabak, ..., Patrick McDonel, Manuel Garber, Mitchell Guttman
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nature
COMMUNICATIONS

ARTICLE

DOI: 10.1038/s41467-018-06961-0 OPEN

Chromatin conformation analysis of primary patient tissue using a low input Hi-C method

Noelia Diaz¹, Kai Kruse¹, Tabea Erdmann², Annette M. Staiger^{3,4,5}, German Ott³, Georg Lenz² & Juan M. Vaquerizas¹

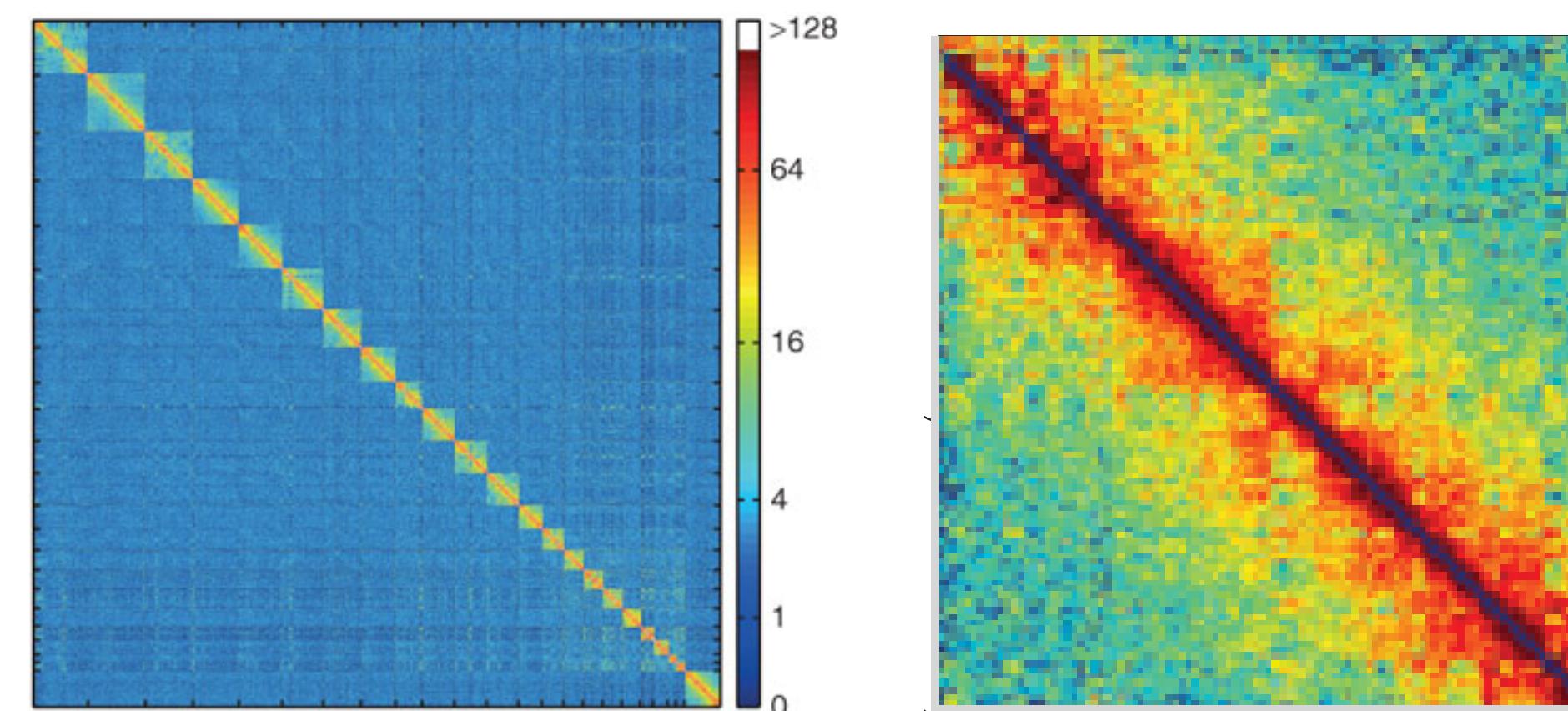
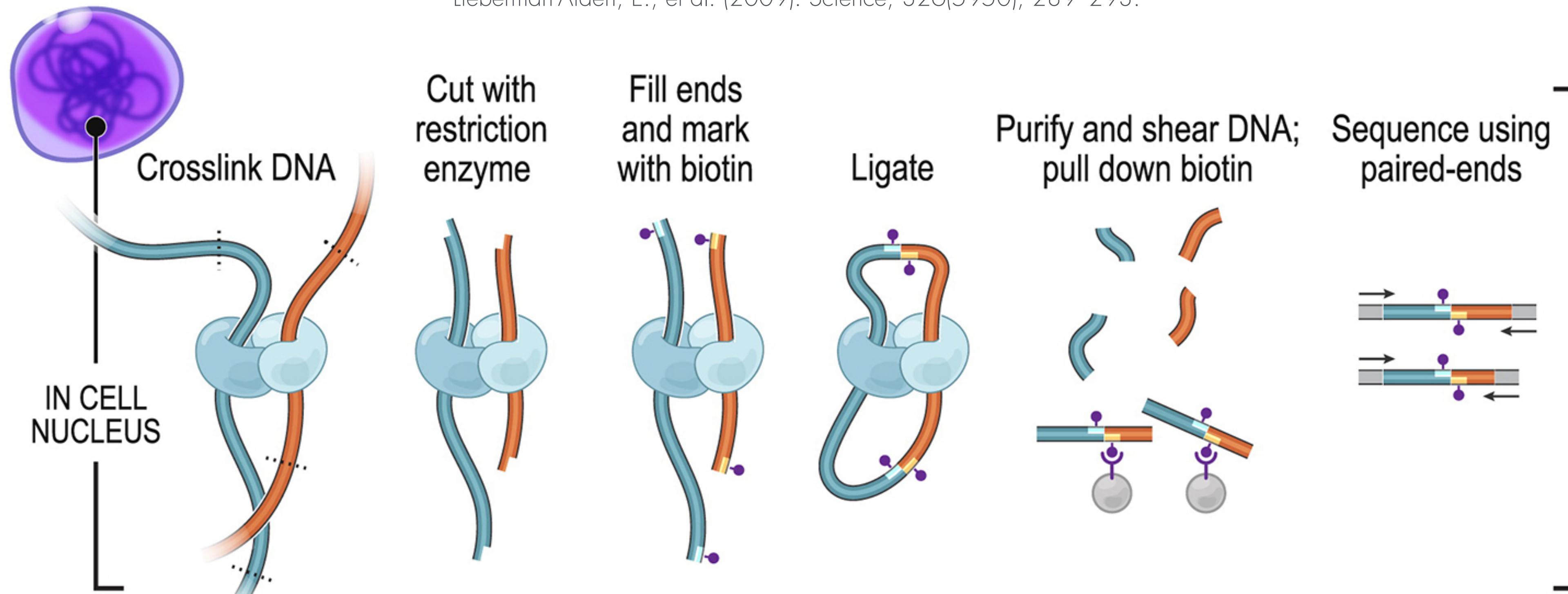
Compartment-dependent chromatin interaction dynamics revealed by liquid chromatin Hi-C

Houda Belaghzal¹, Tyler Borrman², Andrew D. Stephens³, Denis L. Lafontaine¹, Sergey V. Veney¹, Zhiping Weng³, John F. Marko^{3,4}, Job Dekker^{1,5,6#}

Chromosome Conformation Capture

Dekker, J., Rippe, K., Dekker, M., & Kleckner, N. (2002). Science, 295(5558), 1306–1311.

Lieberman-Aiden, E., et al. (2009). Science, 326(5950), 289–293.



Hi-C 3.0

Akgol Oksuz, et al. Nature Methods 2021

ANALYSIS
<https://doi.org/10.1038/s41592-021-01248-7>

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Systematic evaluation of chromosome conformation capture assays

Betul Akgol Oksuz^{1,10}, Liyan Yang^{1,10}, Sameer Abraham², Sergey V. Venev¹, Nils Krietenstein³, Krishna Mohan Parsi^{4,5}, Hakan Ozadam^{1,6}, Marlies E. Oomen¹, Ankita Nand¹, Hui Mao^{4,5}, Ryan M. J. Genga^{4,5}, Rene Maehr^{1,6}, Oliver J. Rando^{1,3}, Leonid A. Mirny^{1,2,7,8}, Johan H. Gibcus^{1,10} and Job Dekker^{1,9,10}

Chromosome conformation capture (3C) assays are used to map chromatin interactions genome-wide. Chromatin interaction maps provide insights into the spatial organization of chromosomes and the mechanisms by which they fold. Hi-C and Micro-C are widely used 3C protocols that differ in key experimental parameters including cross-linking chemistry and chromatin fragmentation strategy. To understand how the choice of experimental protocol determines the ability to detect and quantify aspects of chromosome folding we have performed a systematic evaluation of 3C experimental parameters. We identified optimal protocol variants for either loop or compartment detection, optimizing fragment size and cross-linking chemistry. We used this knowledge to develop a greatly improved Hi-C protocol (Hi-C 3.0) that can detect both loops and compartments relatively effectively. In addition to providing benchmarked protocols, this work produced ultra-deep chromatin interaction maps using Micro-C, conventional Hi-C and Hi-C 3.0 for key cell lines used by the 4D Nucleome project.

Chromosome conformation capture (3C)-based assays¹ have become widely used to generate genome-wide chromatin interaction maps². Analysis of chromatin interaction maps has led to detection of several features of the folded genome. Such features include precise looping interactions (at the 0.1–1 Mb scale) between pairs of specific sites that appear as local dots in interaction maps. Many of such dots represent loops formed by cohesin-mediated loop extrusion that is stalled at convergent CCCTC-binding factor (CTCF) sites^{3,4}. Loop extrusion also produces other features in interaction maps such as stripe-like patterns anchored at specific sites that block loop extrusion. The effective depletion of interactions across such blocking sites leads to domain boundaries (insulation). At the megabase scale, interaction maps of many organisms including mammals display checkerboard patterns that represent the spatial compartmentalization of two main types of chromatin: active and open A-type chromatin domains, and inactive and more closed B-type chromatin domains⁵.

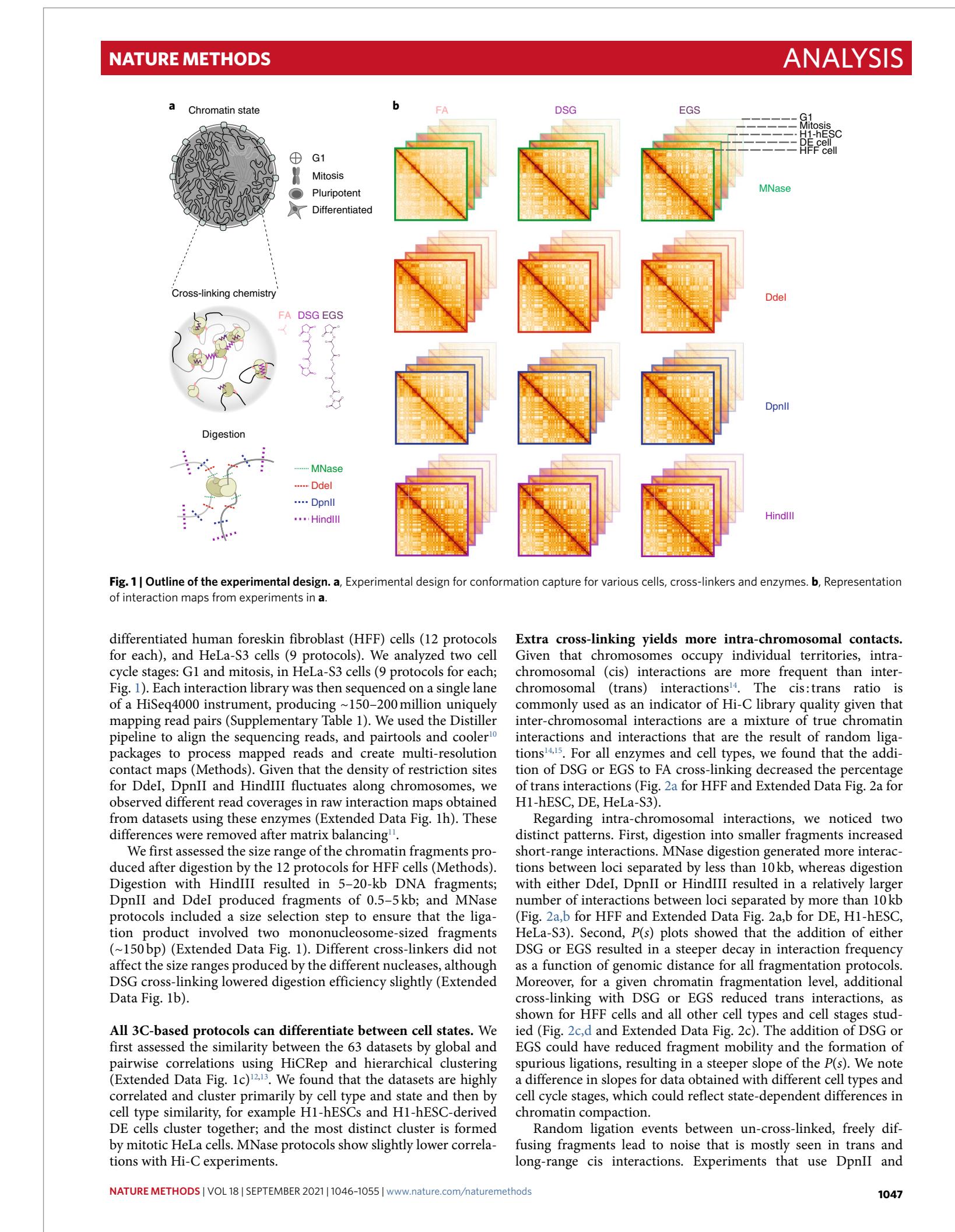
The Hi-C protocol has evolved over the years. While initial protocols used restriction enzymes such as HindIII that produces relatively large fragments of several kilobases⁶, over the last 5 years Hi-C using DpnII or MboI digestion has become the protocol of choice for mapping chromatin interactions at kilobase resolution⁷. More recently, Micro-C, which uses MNase instead of restriction enzymes as well as a different cross-linking protocol, was shown to allow generation of nucleosome-level interaction maps^{8,9}. It is critical to ascertain how key parameters of these 3C-based methods, including cross-linking and chromatin fragmentation, quantitatively influence the detection of chromatin interaction frequencies and the detection of different chromosome folding features that range from local looping between small intra-chromosomal (*cis*) elements to global compartmentalization of megabase-sized domains. Here, we systematically assessed how different cross-linking and fragmentation methods yield quantitatively different chromatin interaction maps.

Results

We explored how two key parameters of 3C-based protocols, cross-linking and chromatin fragmentation, determine the ability to quantitatively detect chromatin compartment domains and loops. We selected three cross-linkers widely used for chromatin: 1% formaldehyde (FA), conventional for most 3C-based protocols; 1% FA followed by incubation with 3 mM disuccinimidyl glutarate (the FA + DSG protocol); and 1% FA followed by incubation with 3 mM ethylene glycol bis(succinimidylsuccinate) (the FA + EGS protocol) (Fig. 1a). We selected four different nucleases for chromatin fragmentation: MNase, Ddel, DpnII and HindIII, which fragment chromatin in sizes ranging from single nucleosomes to multiple kilobases. Combined, the three cross-linking and four fragmentation strategies yield a matrix of 12 distinct protocols (Fig. 1b). To determine how performance of these protocols varies for different states of chromatin we applied this matrix of protocols to multiple cell types and cell cycle stages. We analyzed four different cell types: pluripotent H1 human embryonic stem cells (H1-hESCs), differentiated endoderm (DE) cells derived from H1-hESCs, fully

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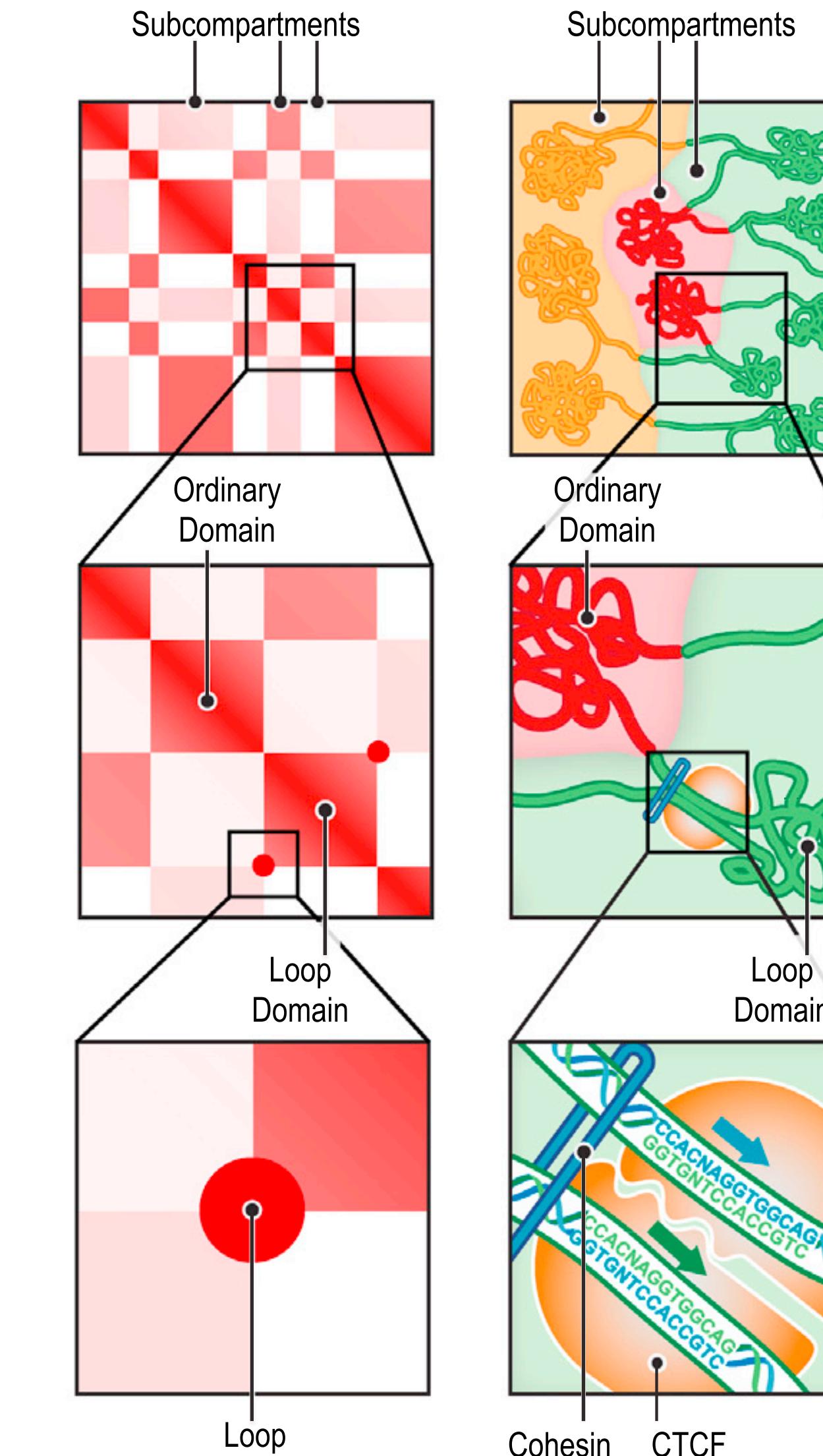
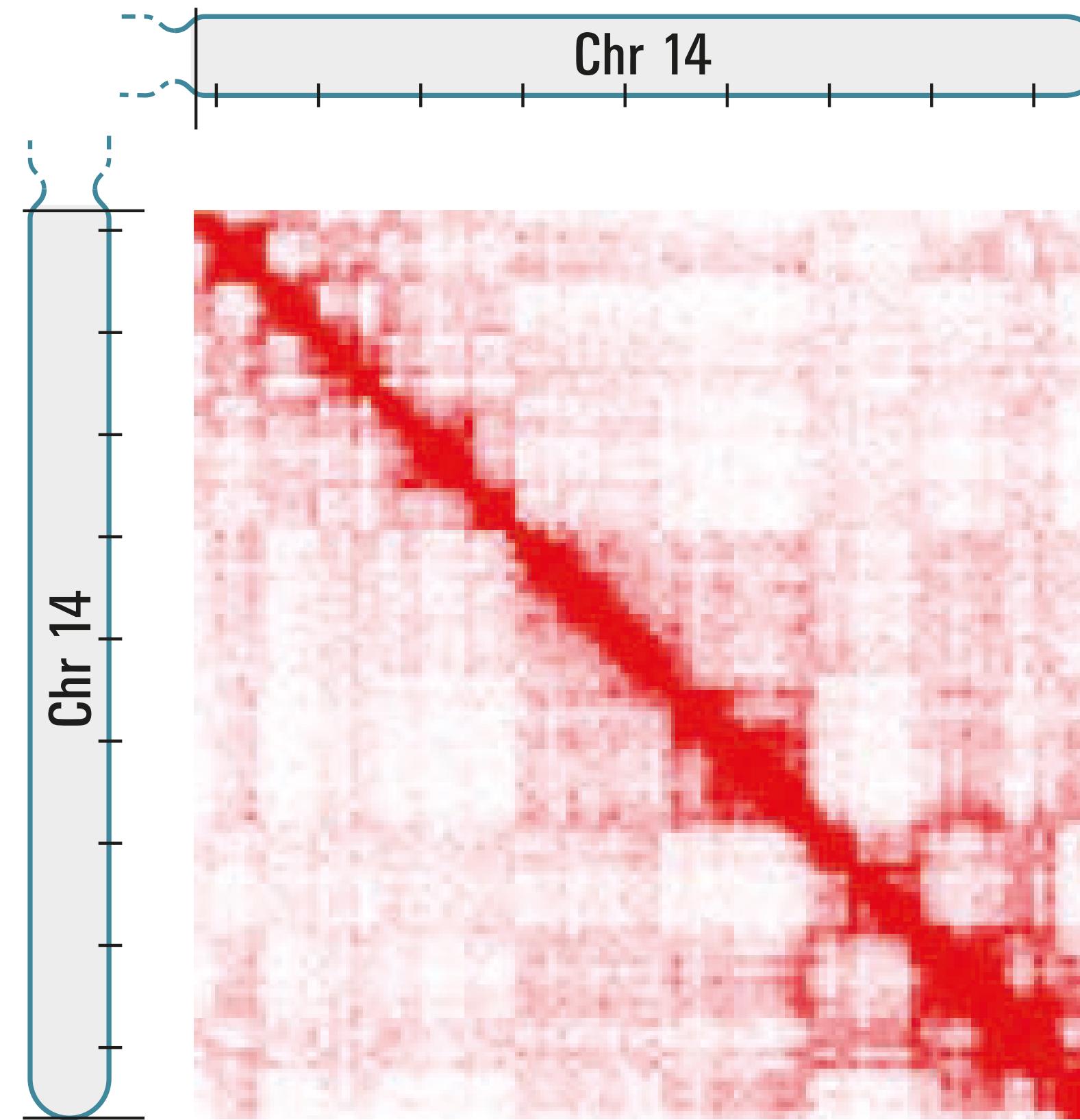
NATURE METHODS | VOL 18 | SEPTEMBER 2021 | 1046–1055 | www.nature.com/naturemethods



Hierarchical genome organisation

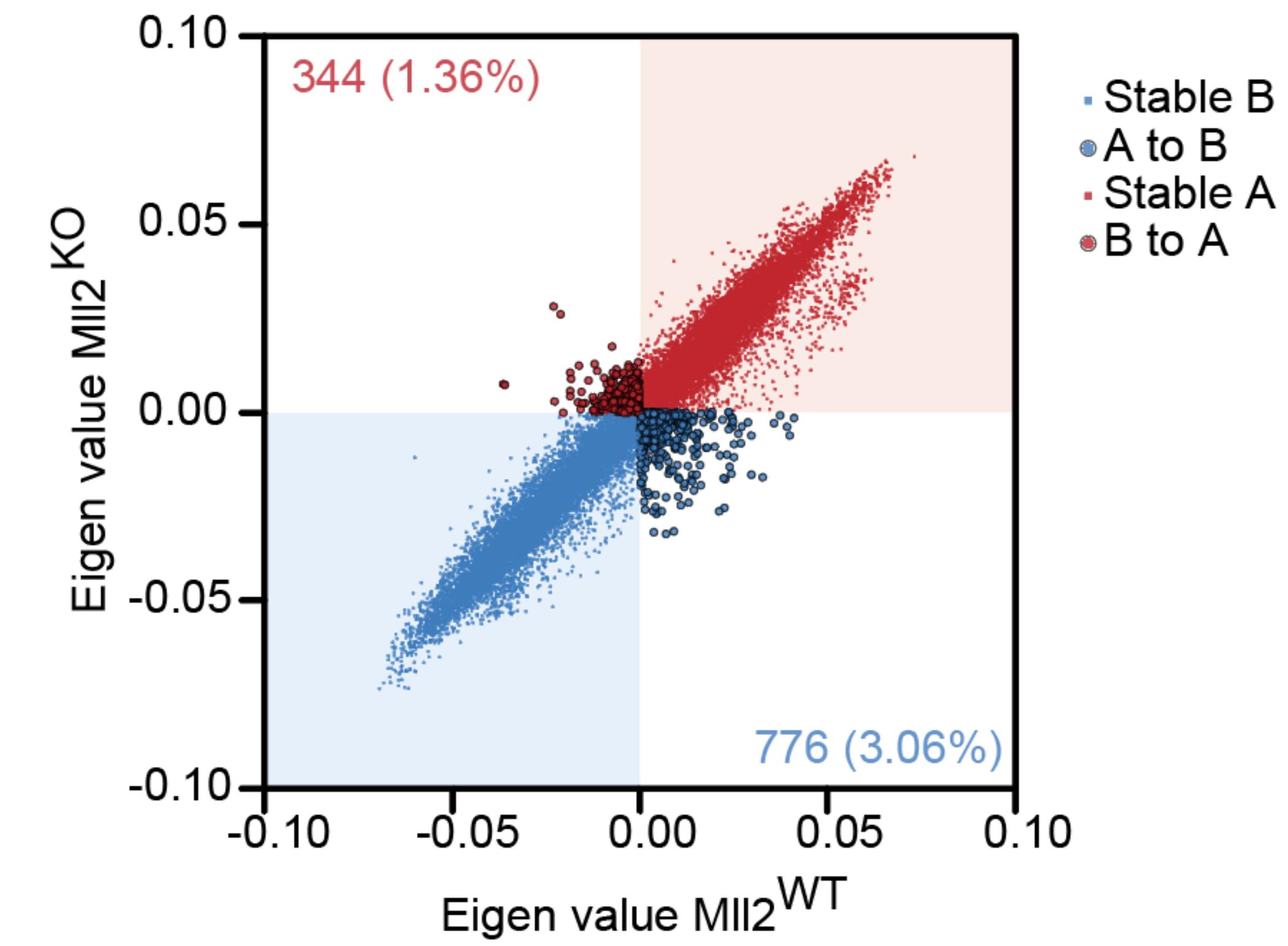
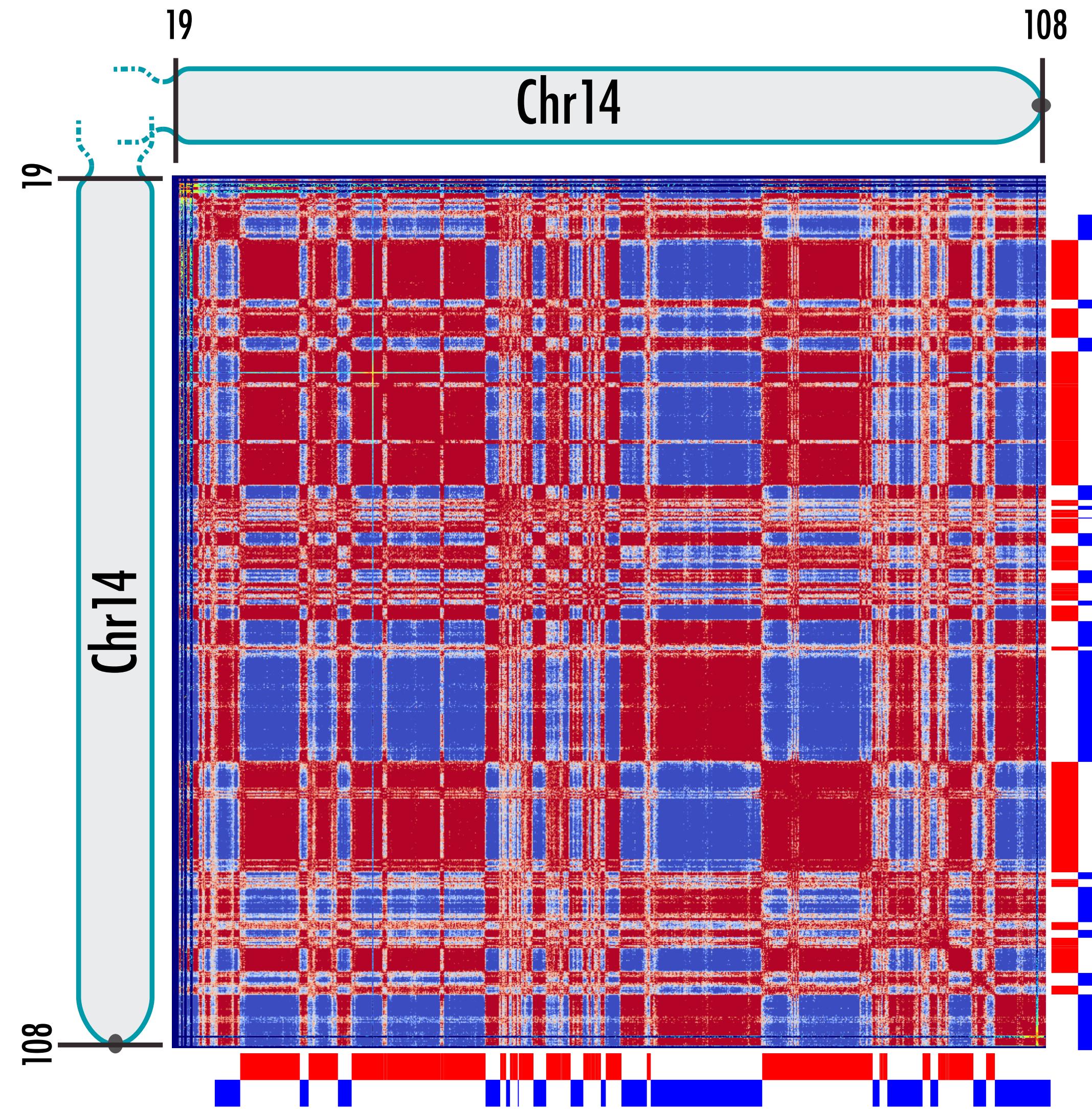
Lieberman-Aiden, E., et al. (2009). Science, 326(5950), 289–293.

Rao, S. S. P., et al. (2014). Cell, 1–29.



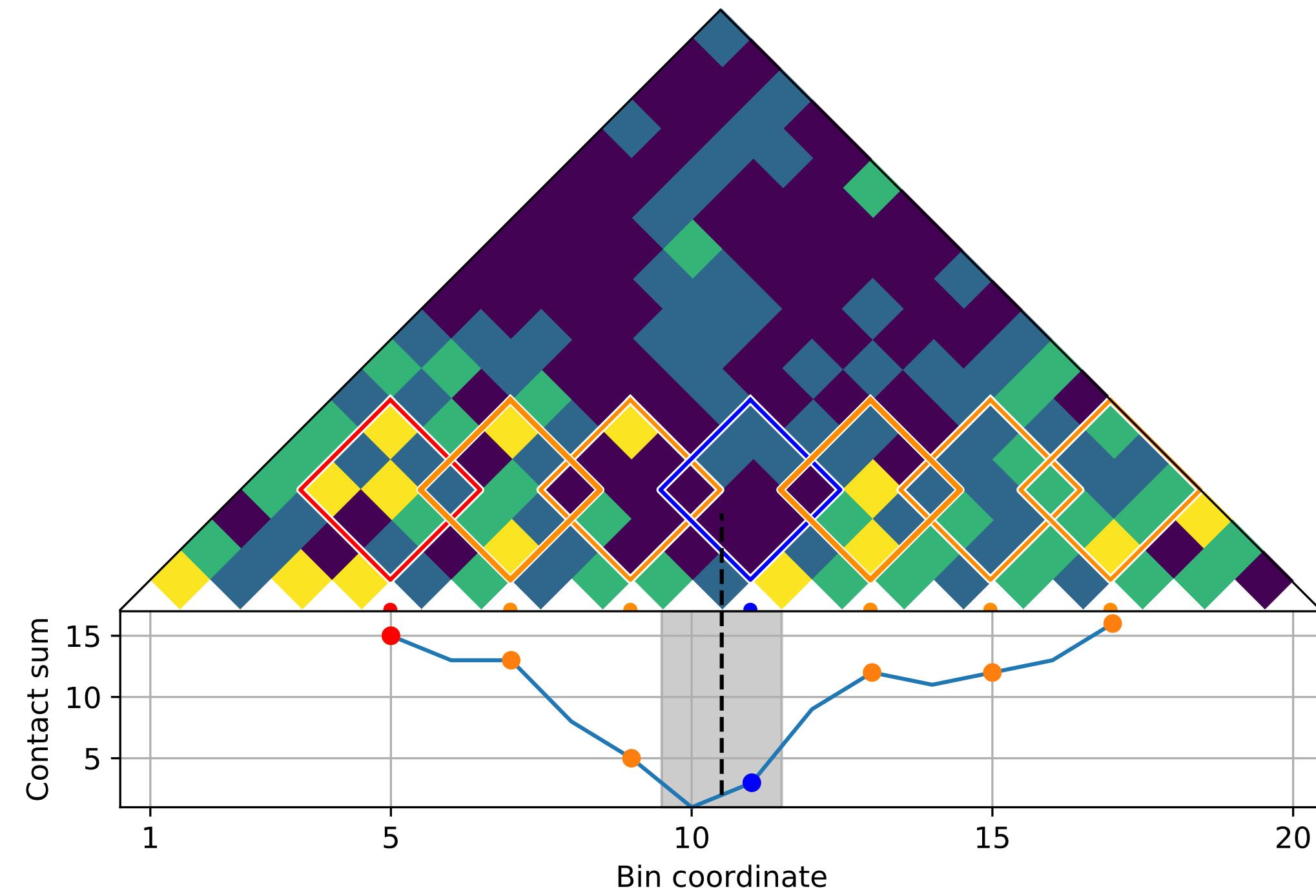
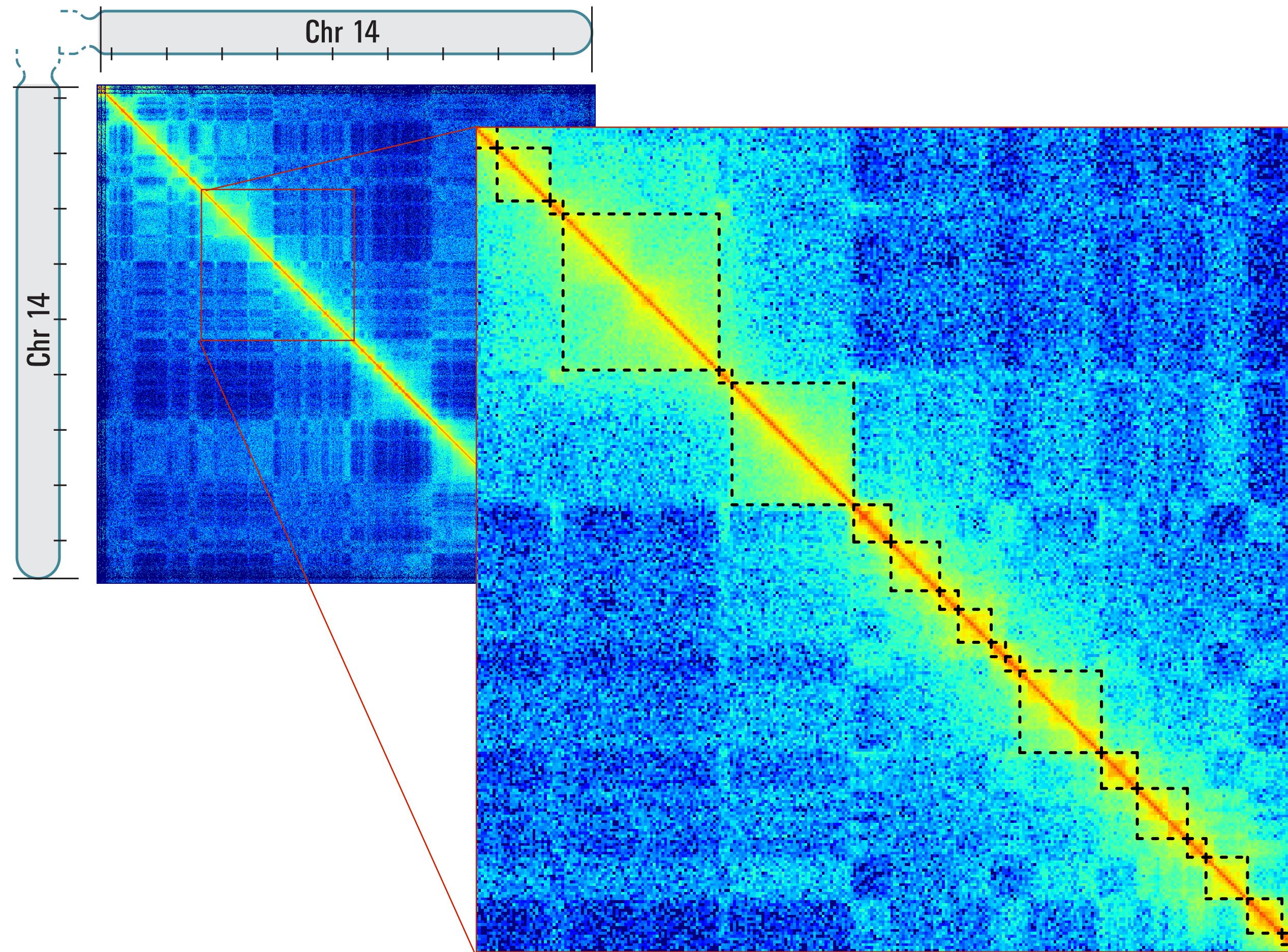
A/B Compartiment

Chromosome 14



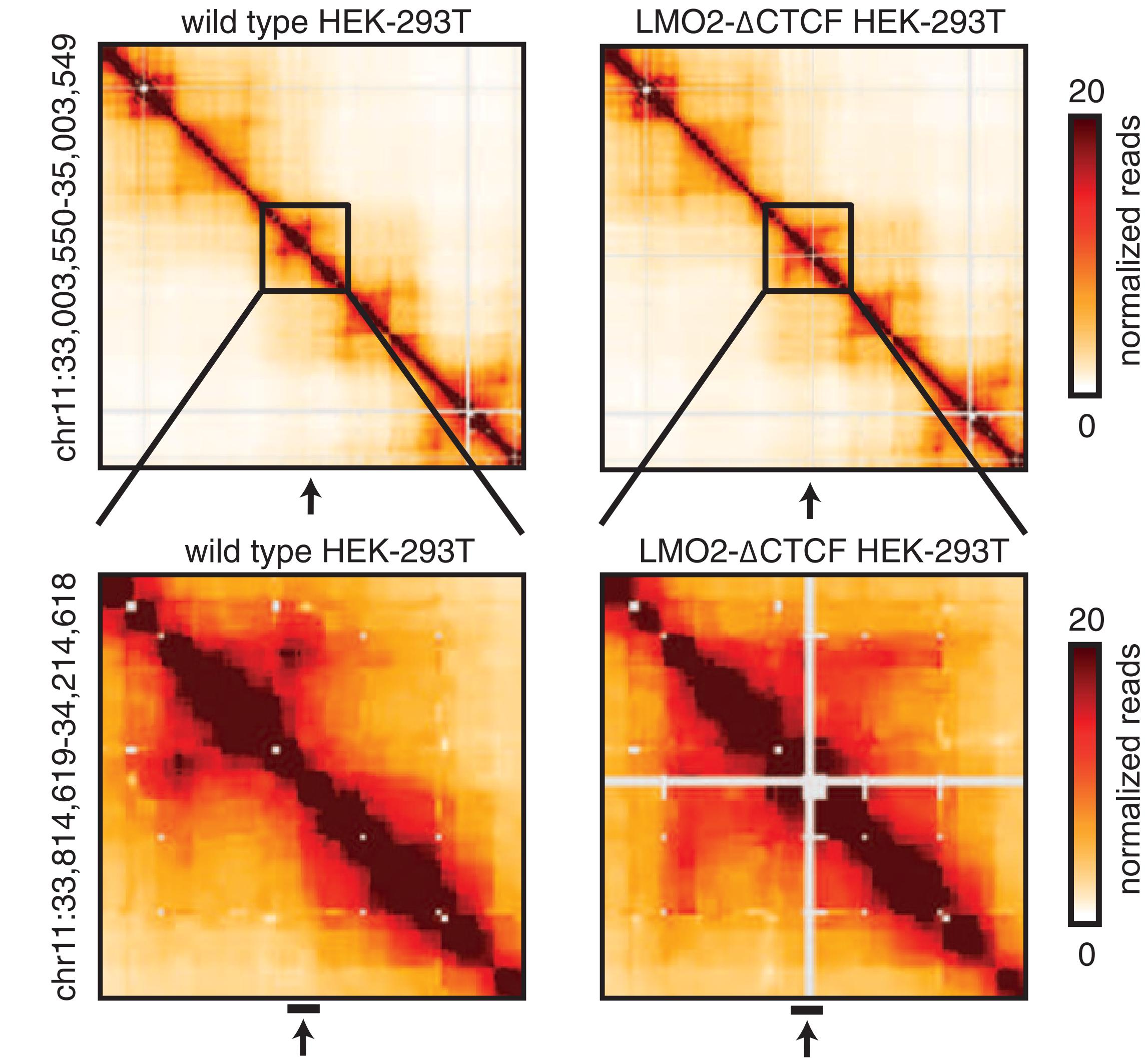
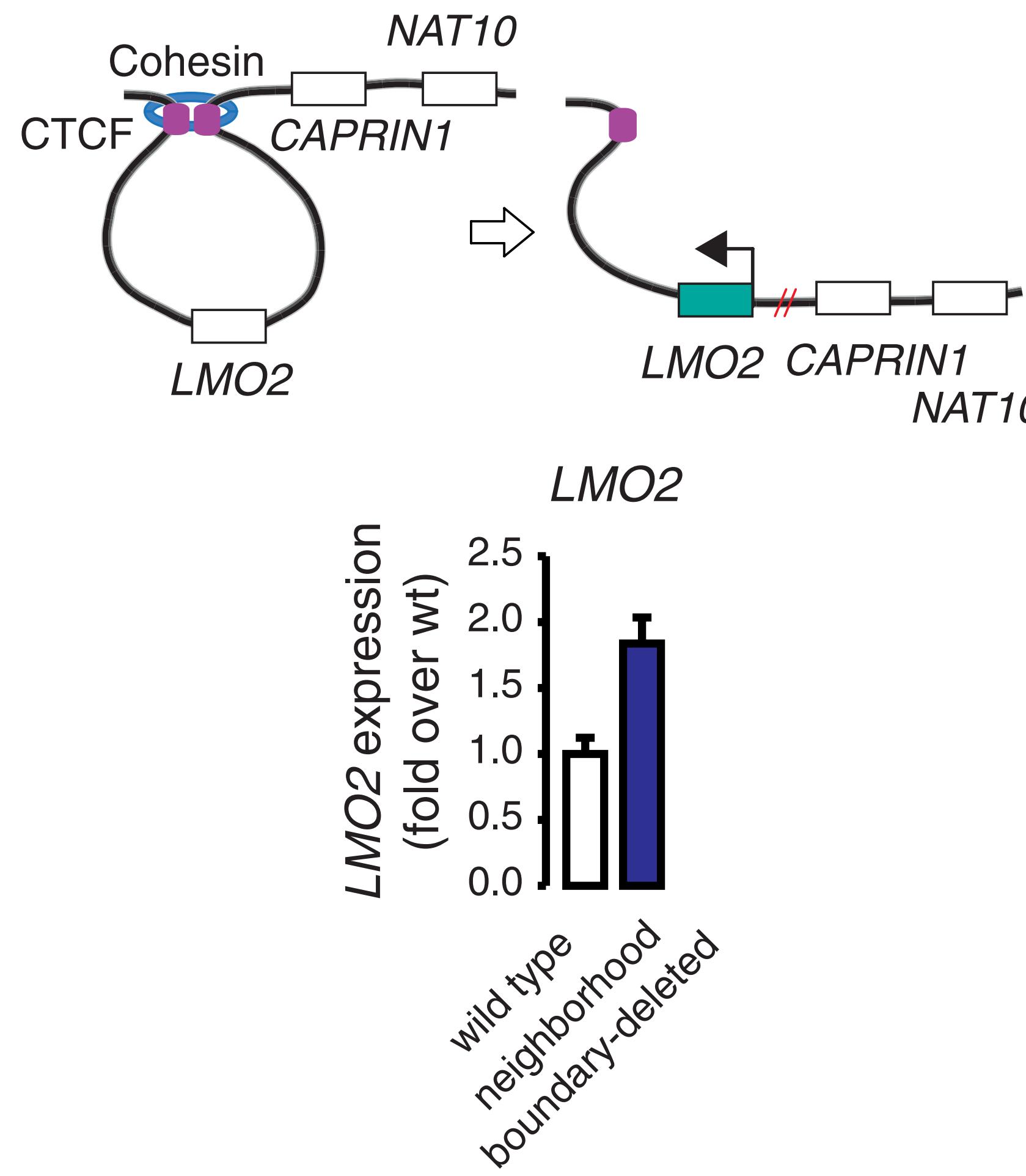
TADs

Chromosome 14



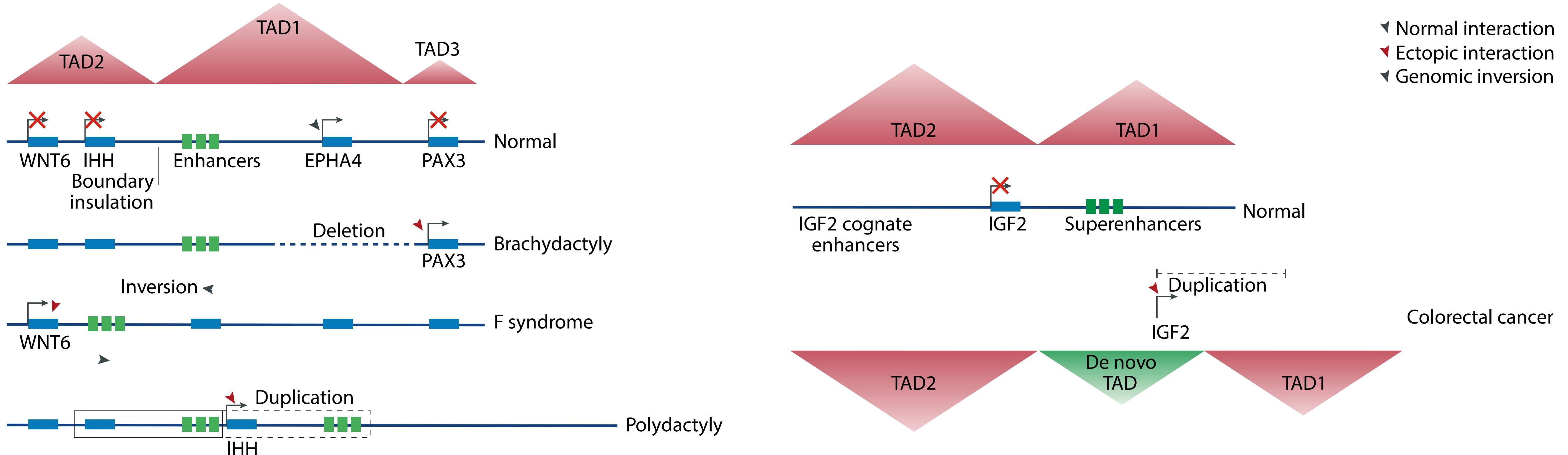
TADs are functional units

Hnisz, D., et al. (2016). Science



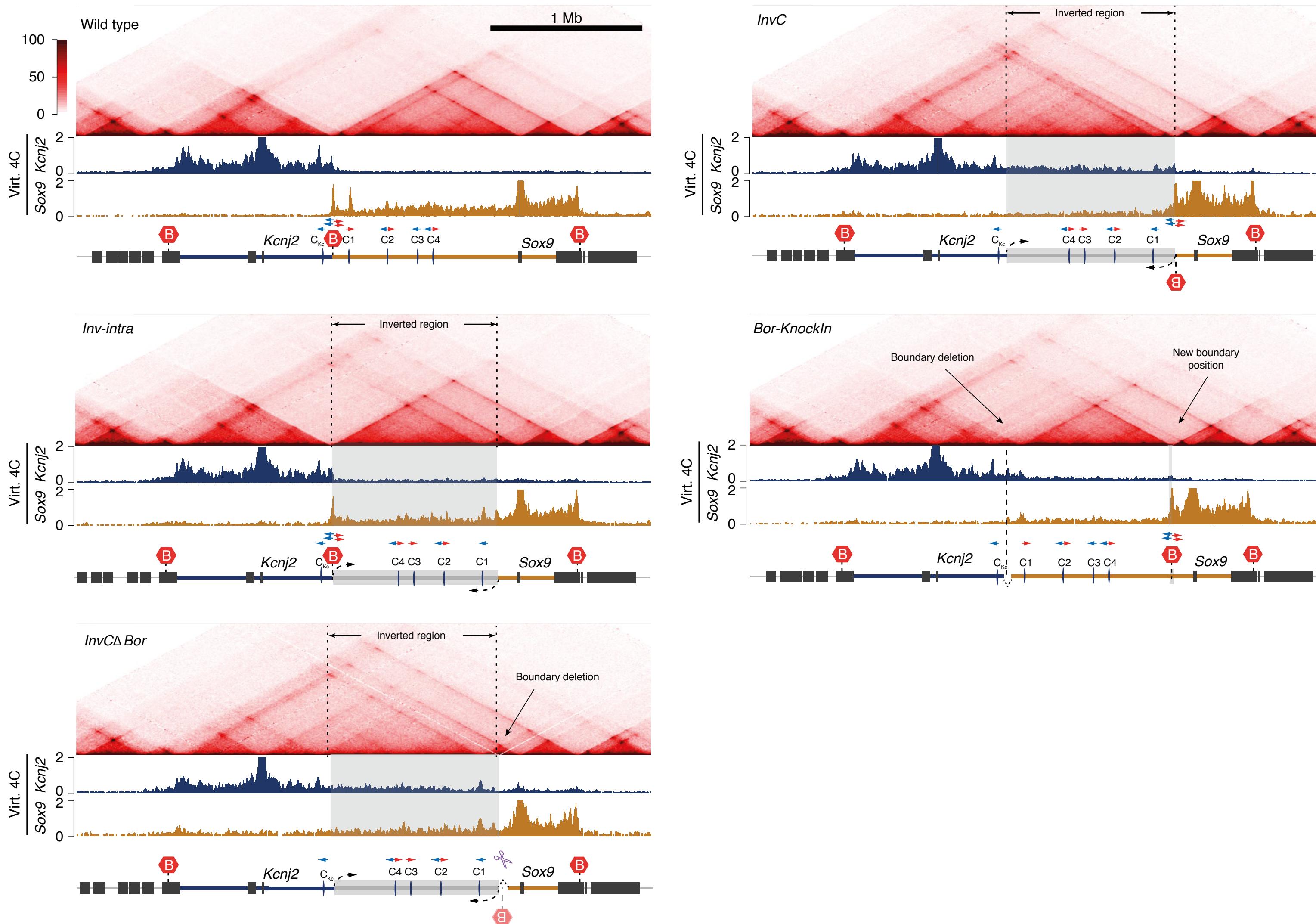
TADs are functional units

Figure adapted from Hui Zheng and Wei Xie. *Nature Reviews Molecular Cell Biology* (2019)



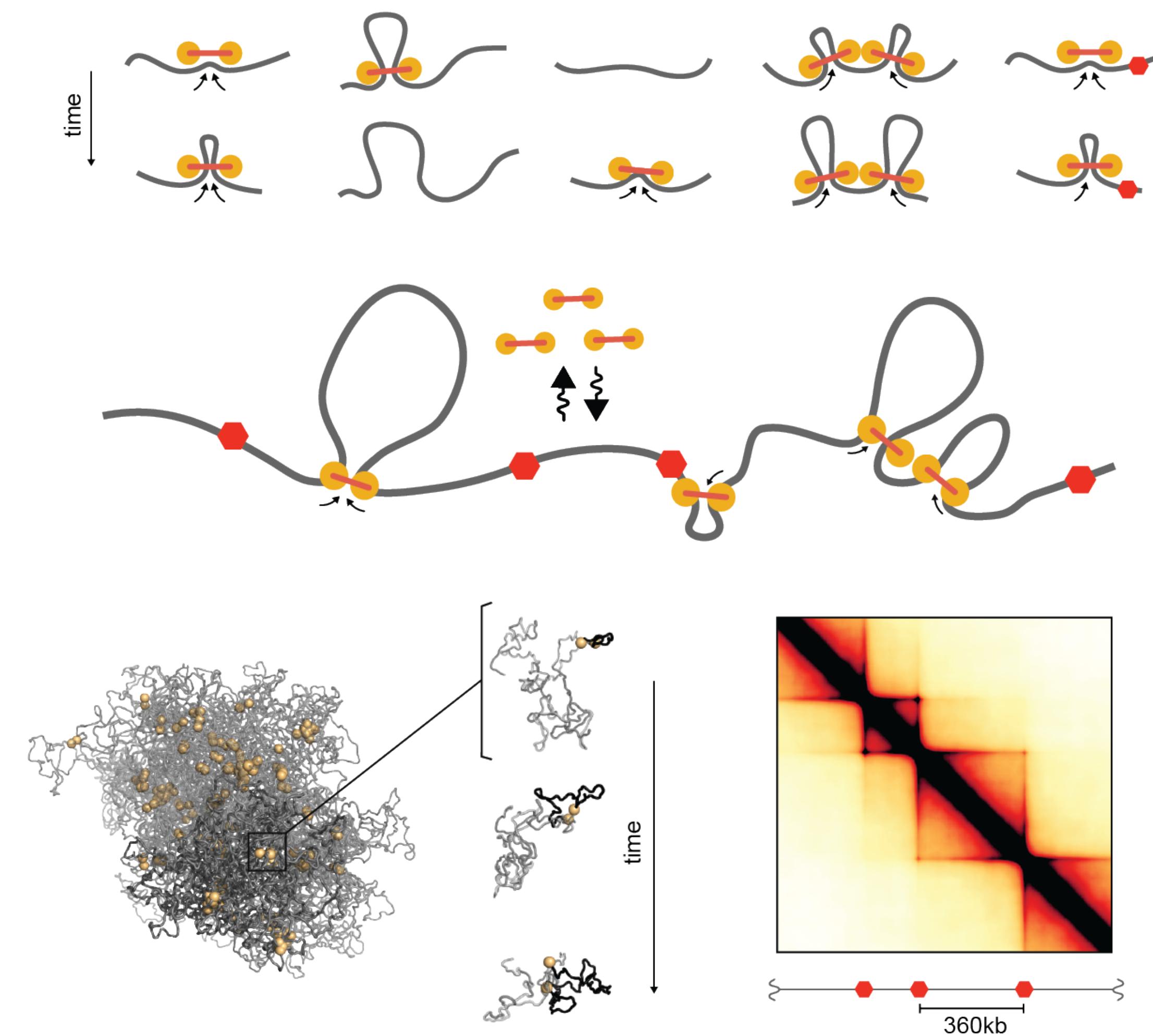
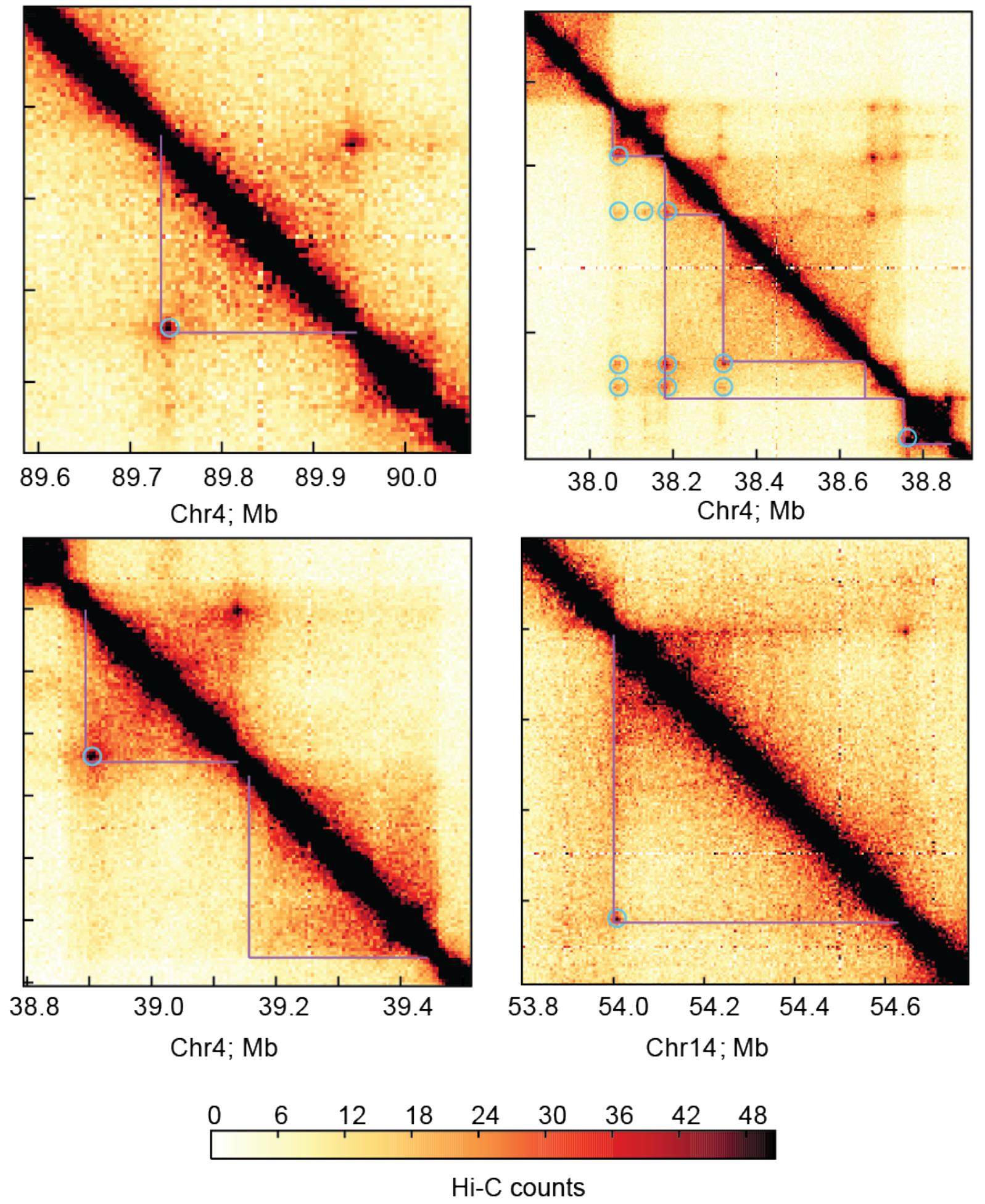
TADs are functional units

Despang, et al. (2019). Nature Genetics 51, 1263–1271 (2019)



Loop-extrusion as a TAD forming mechanism

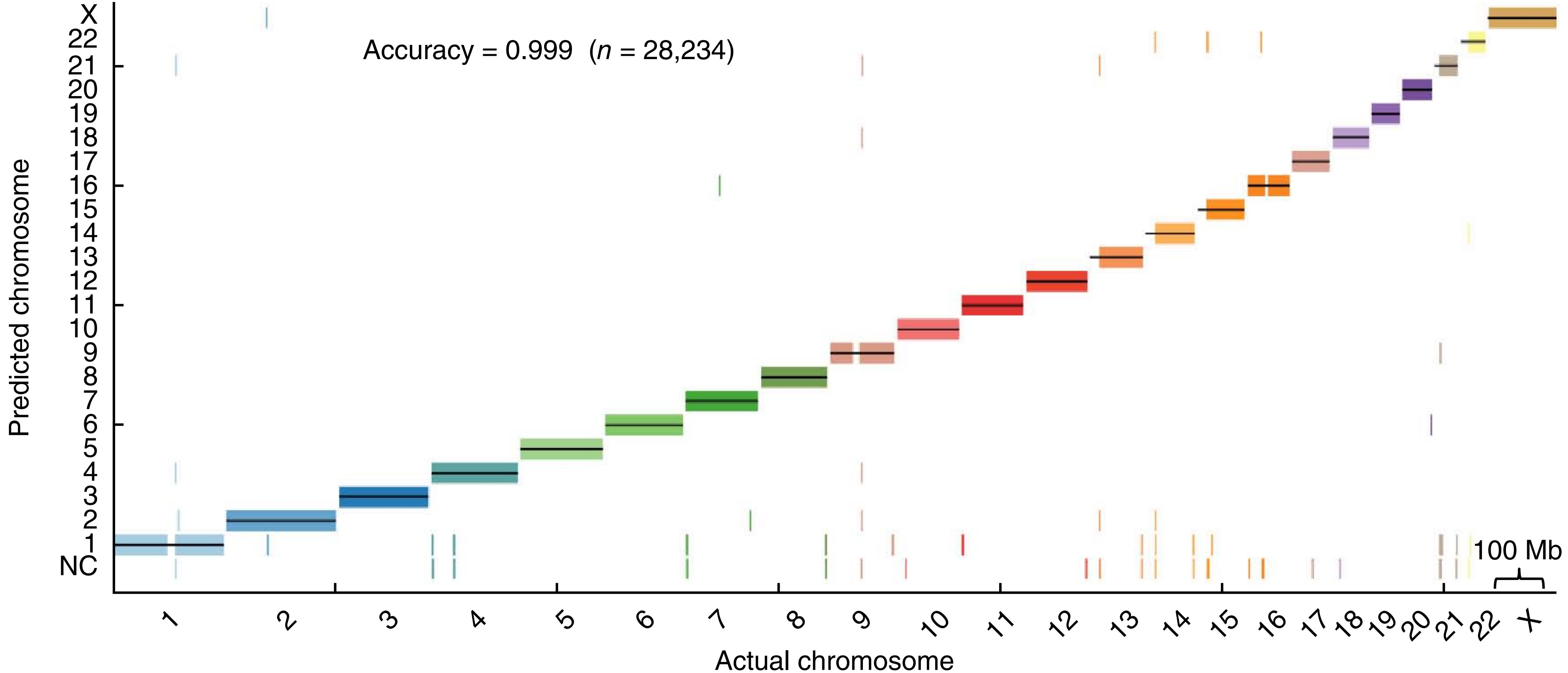
Fudenberg, G., Imakaev, M., Lu, C., Goloborodko, A., Abdennur, N., & Mirny, L. A. (2018).
Cold Spring Harb Symp Quant Biol 2017. 82: 45-55



SIDE EFFECTS

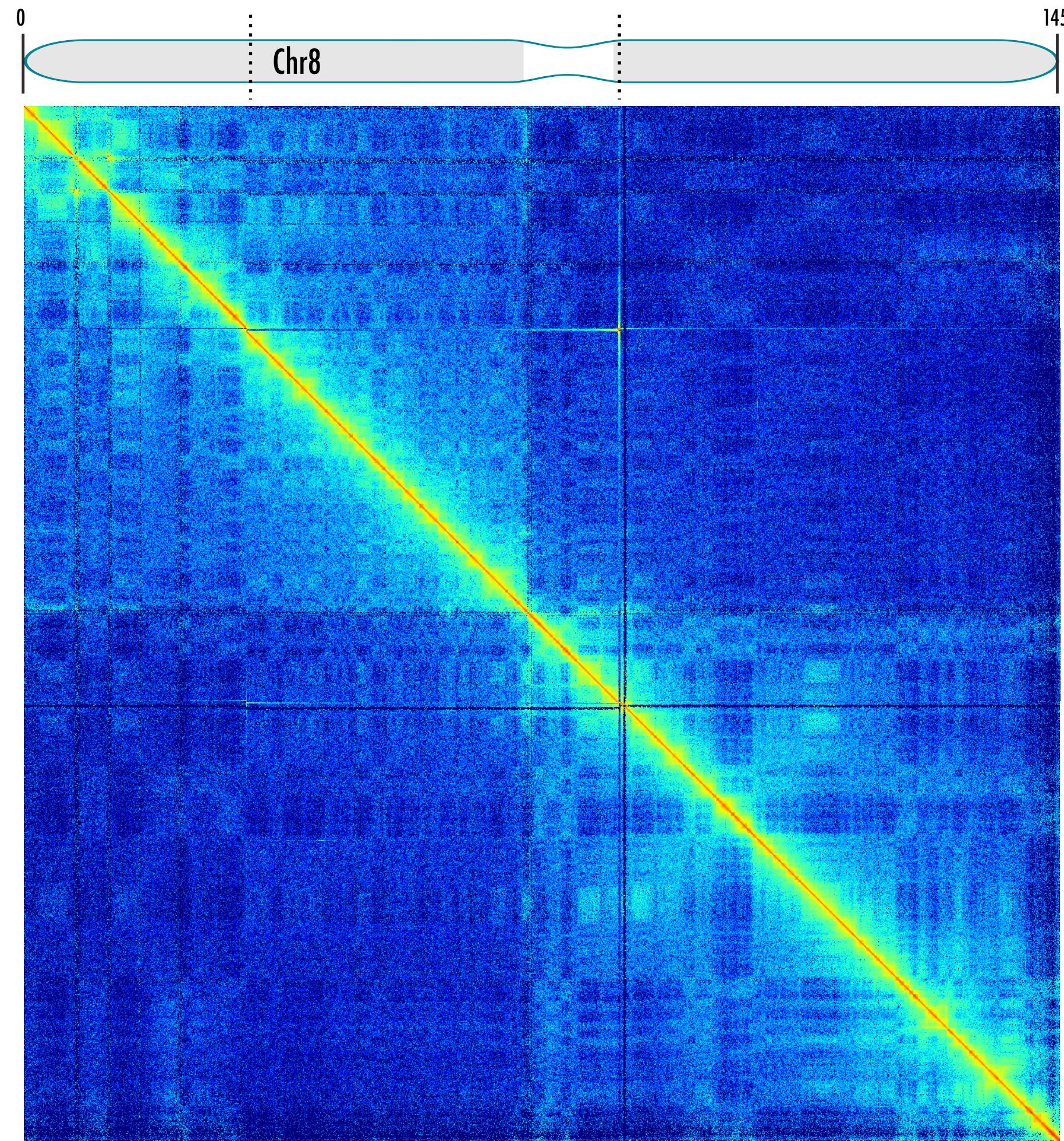
Hi-C for de-novo assembly

Kaplan, N., & Dekker, J. (2013). Nature Biotechnology, 31(12), 1143–1147.

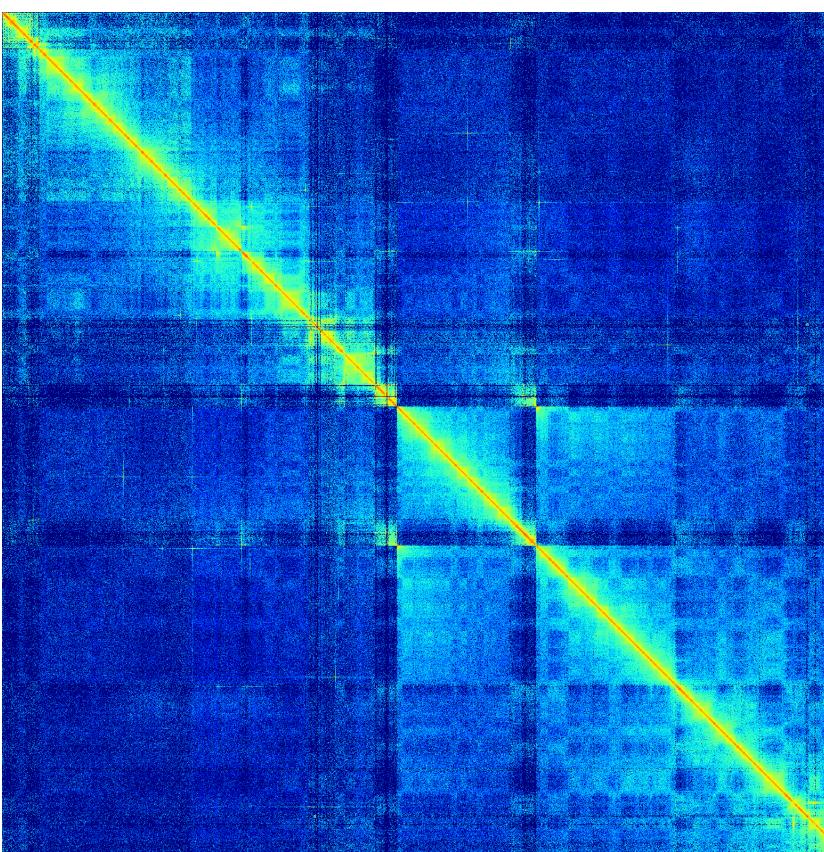


Assembly error detection

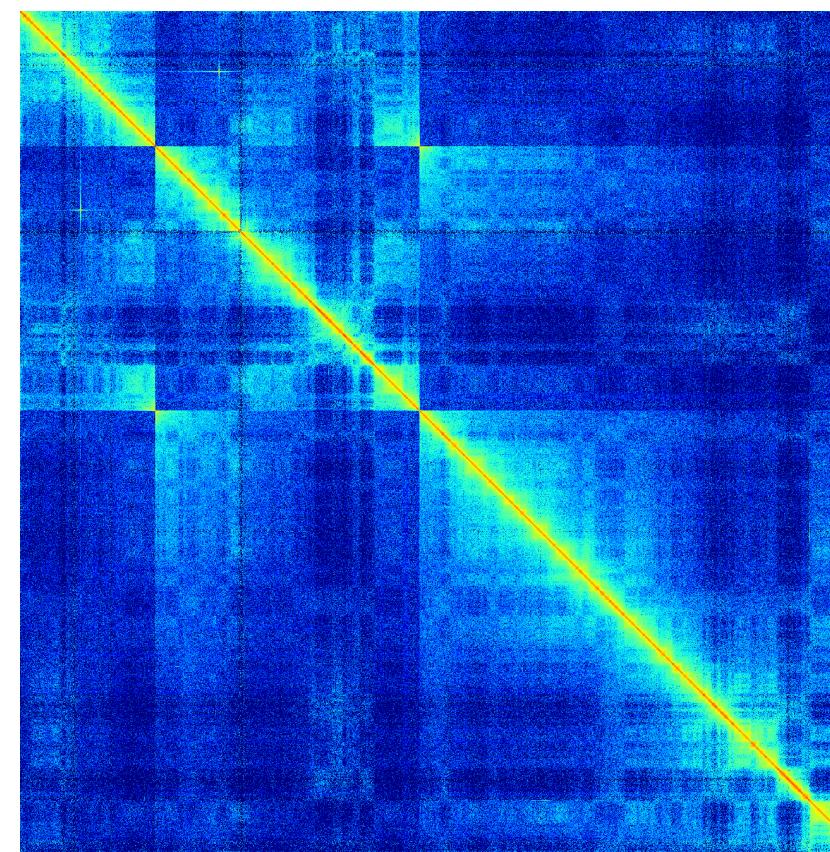
Chromosome 8 Gorilla



Chr 7



Chr 12



GGO8 has an inversion of the region corresponding to HSA8:30.0-86.9Mb
Aylwyn Scally (Department of Genetics, University of Cambridge)

Hi-C for meta genomics

Beitel, C. W., Froenicke, L., Lang, J. M., Korf, I. F., Michelmore, R. W., Eisen, J. A., & Darling, A. E. (2014). Strain- and plasmid-level deconvolution of a synthetic metagenome by sequencing proximity ligation products. doi:10.7287/peerj.preprints.260v1

Romain Koszul

