NATURE METHODS | ADVANCE ONLINE PUBLICATION

HiChIP: efficient and sensitive analysis of protein-directed genome architecture

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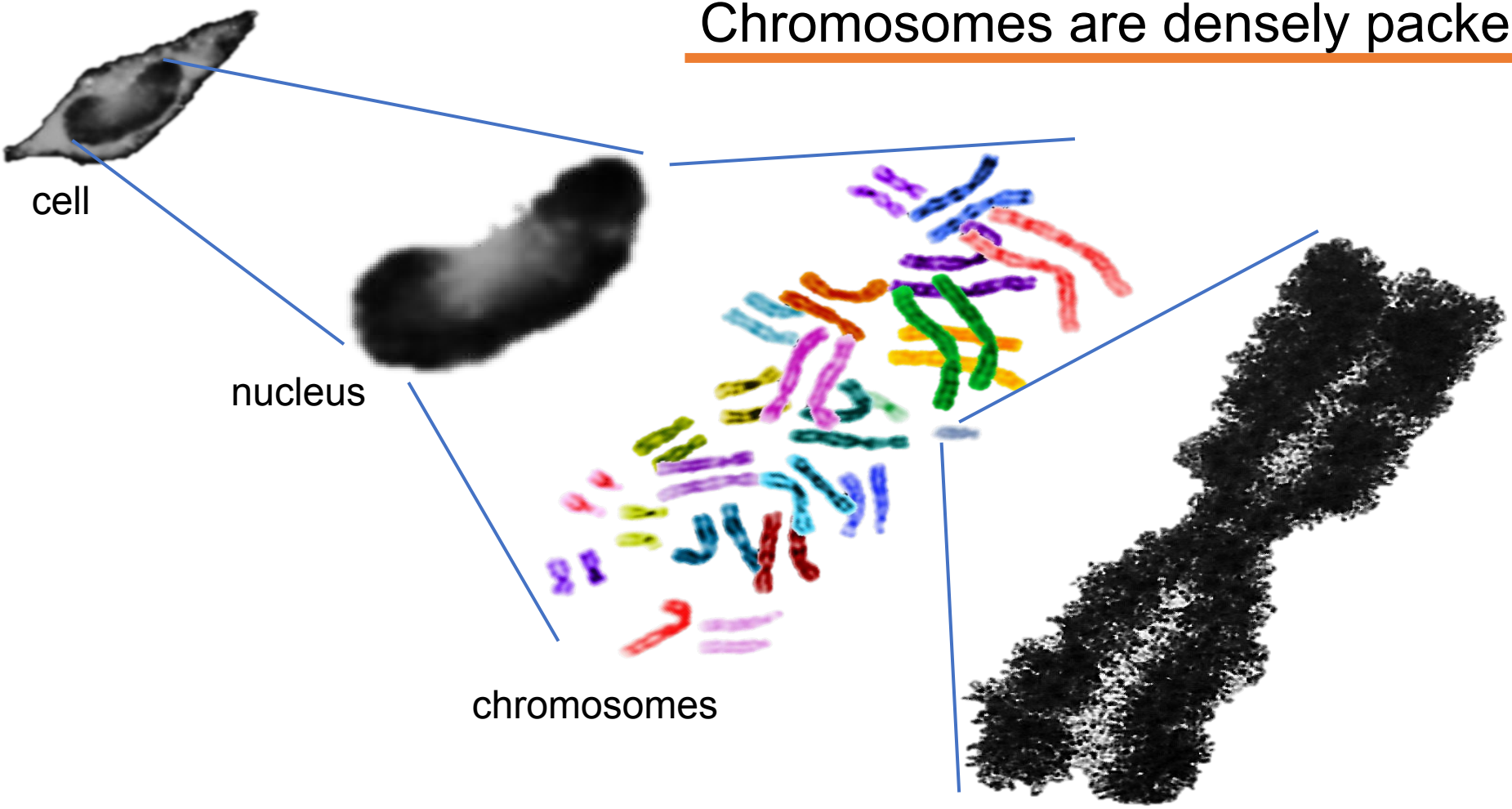
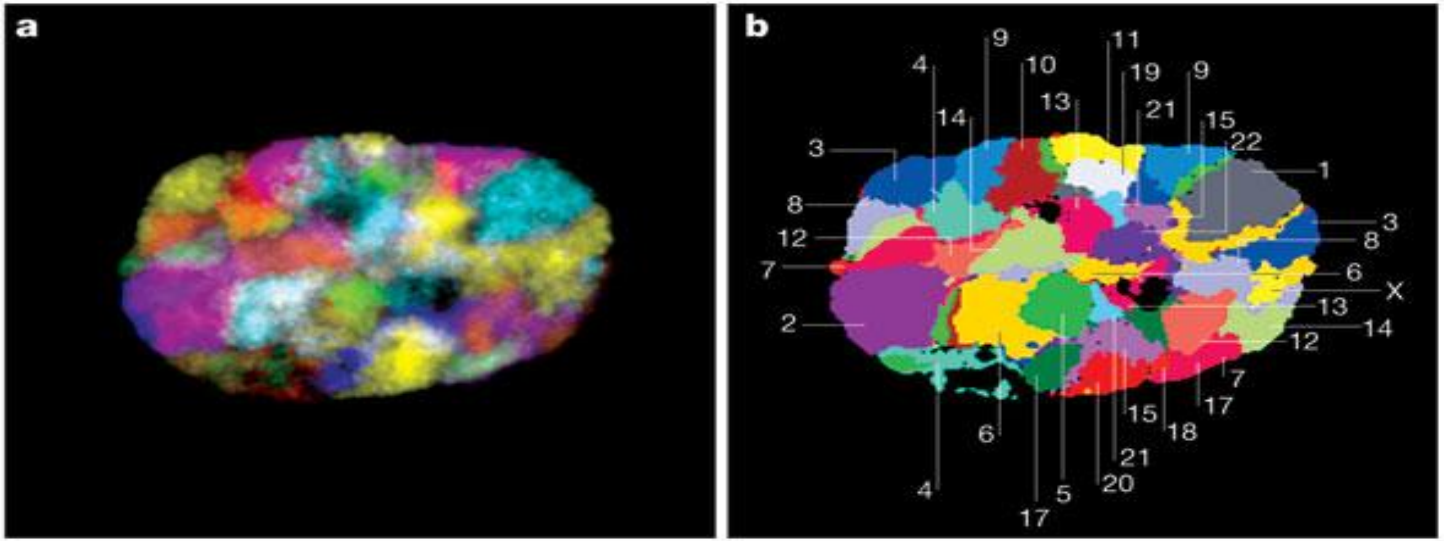
Hazreen Abdul Majid, Noora J. Al-Muftah, Ibrahim Kurt

2017 BST281

Chromosomes are densely packed



Nuclear landscape is revealed by 3D-FISH

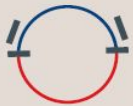


Speicher MR, and Carter NP. (2005). The new cytogenetics: blurring the boundaries with molecular biology. *Nat. Rev. Genet.* **6**: 782–92.

Chromosome Conformation Capture (CCC) methods evolved in time

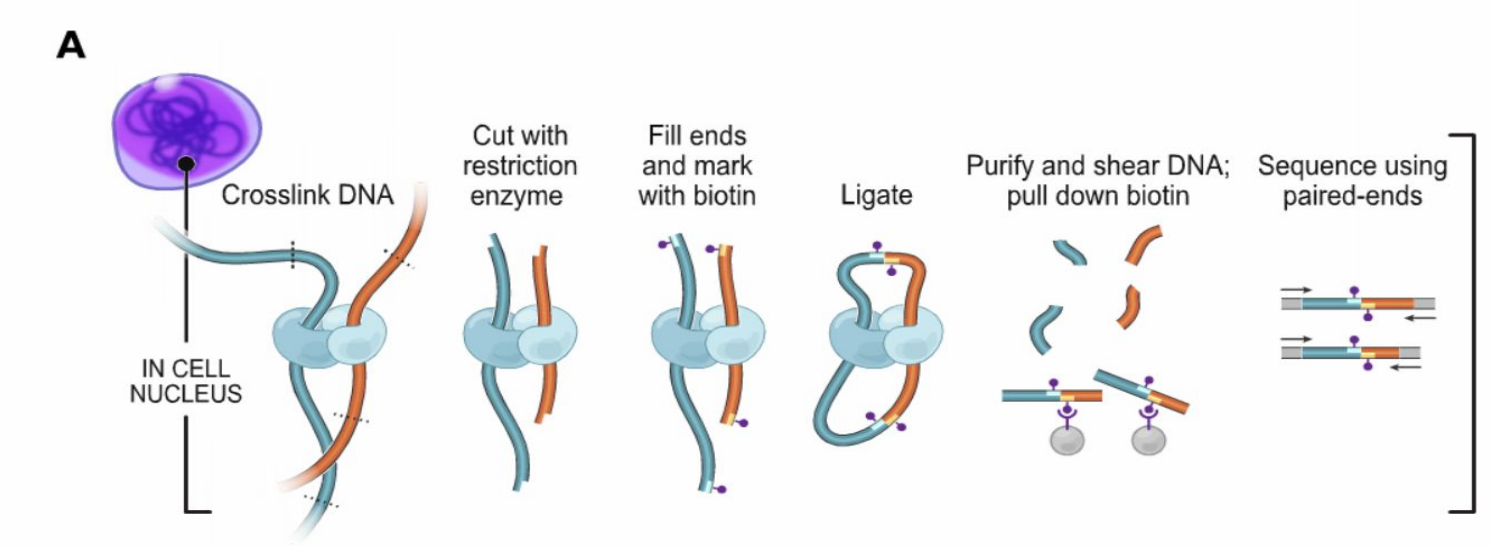
a 3C: converting chromatin interactions into ligation products

b Ligation product detection methods

3C	4C	5C
One-by-one All-by-all	One-by-all	Many-by-many
		
PCR or sequencing	Inverse PCR sequencing	Multiplexed LMA sequencing

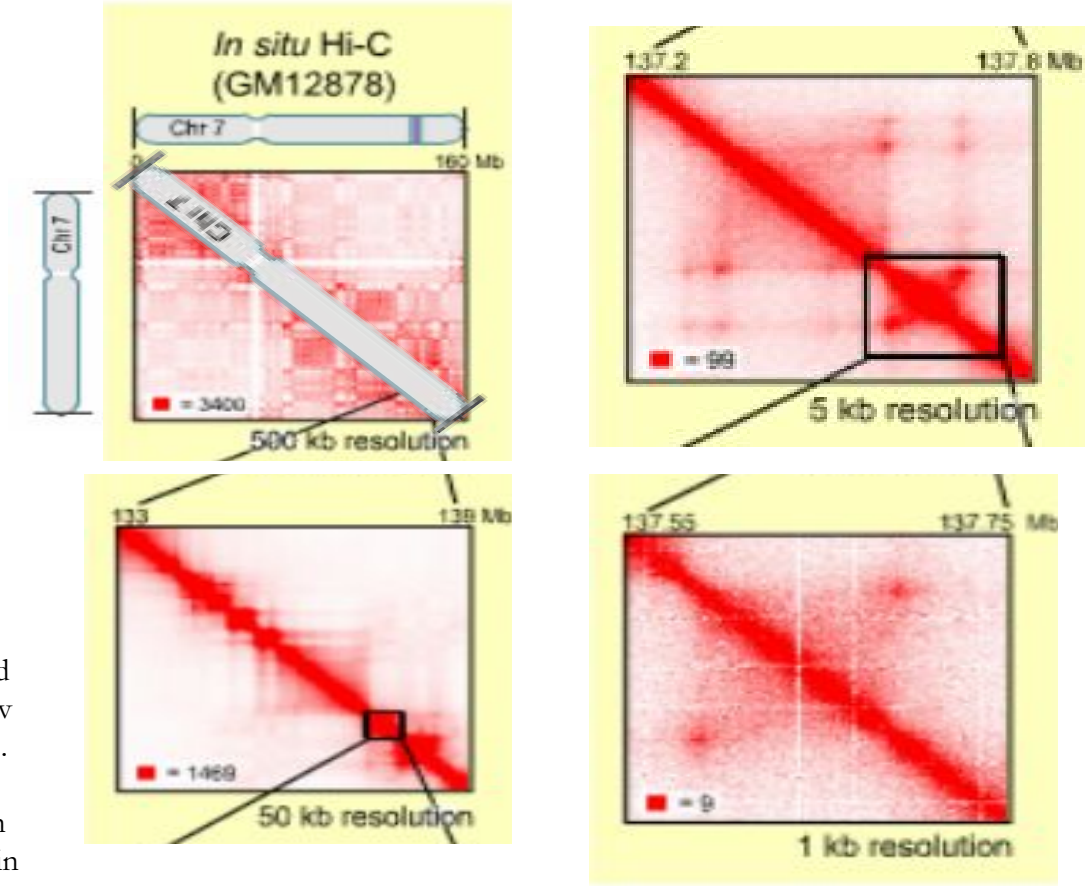
Dekker et al. 2013 Nat. Rev. Gen.

The Hi-C method was the first unbiased and genome-wide adaptation of 3C



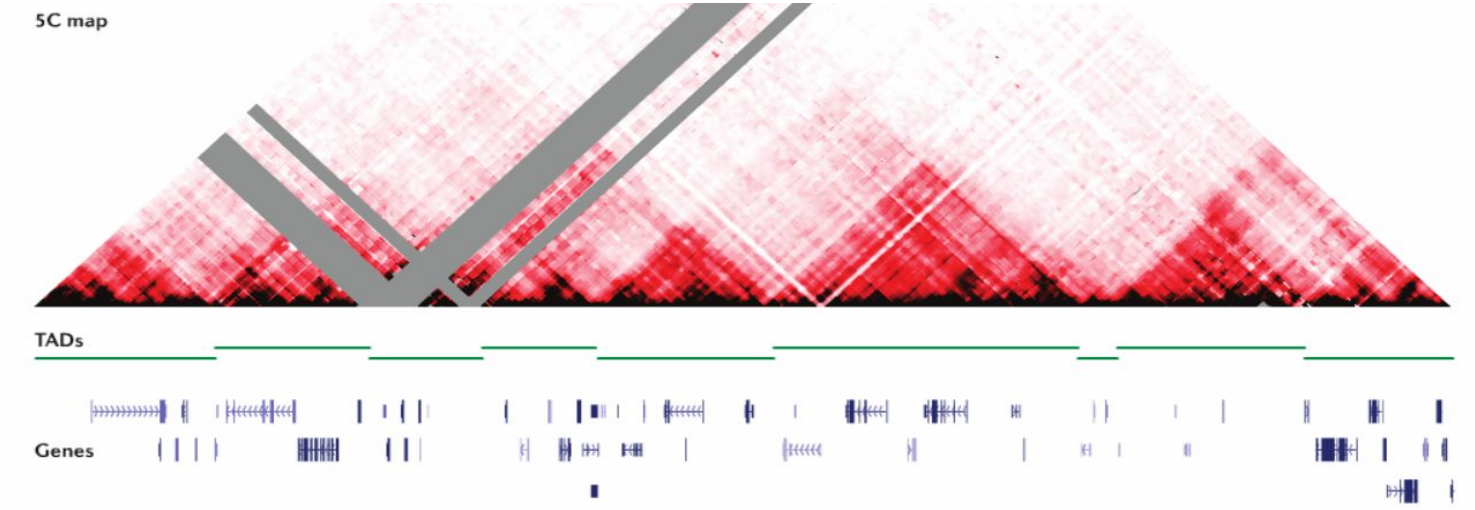
Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, *et al.* (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**: 1665–80.

HiC reveals the square-like intra-chromosomal interaction patterns



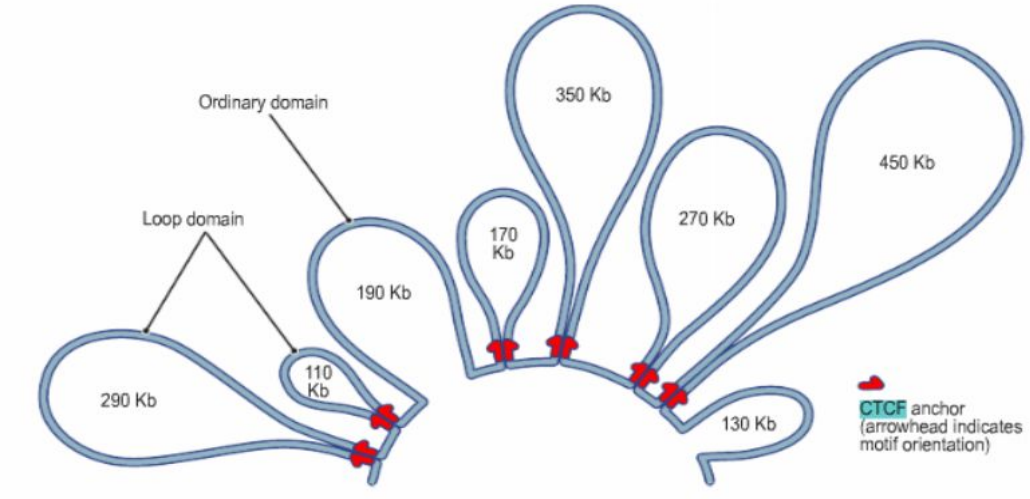
Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, *et al.* (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**: 1665–80.

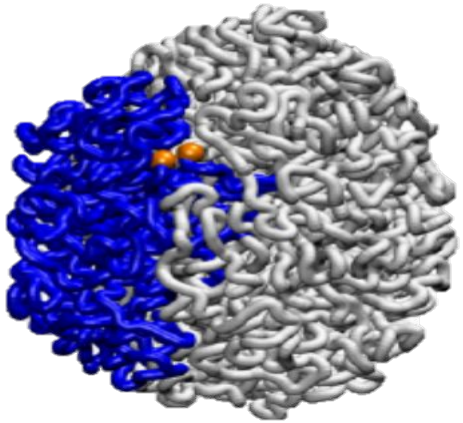
Topologically associated domains reveal the borders of loops



Dekker J, Marti-Renom MA, and Mirny LA. (2013). Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data.*Nat. Rev. Genet.* **14**: 390–403.

In a perfect 2D-world, interphase chromatin might look like this

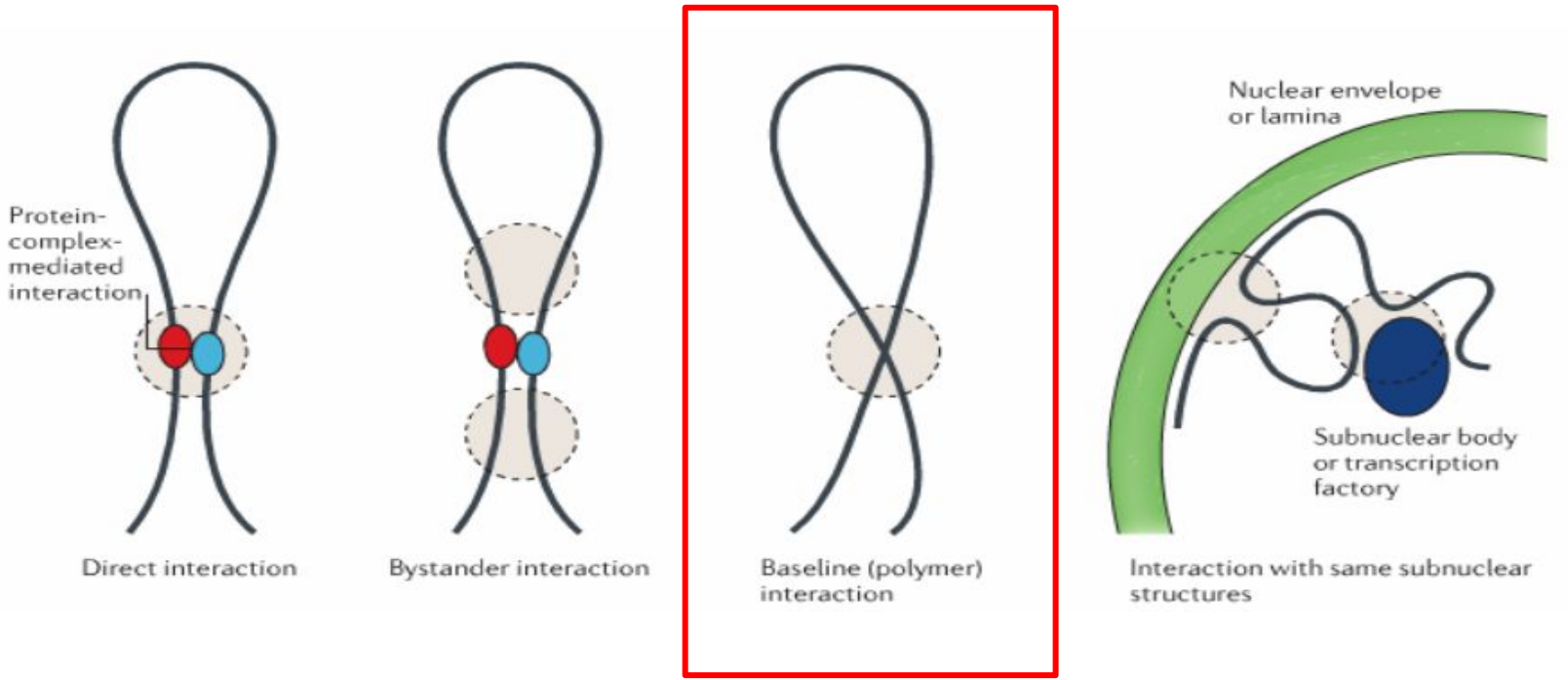




Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, *et al.* (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**: 1665–80.

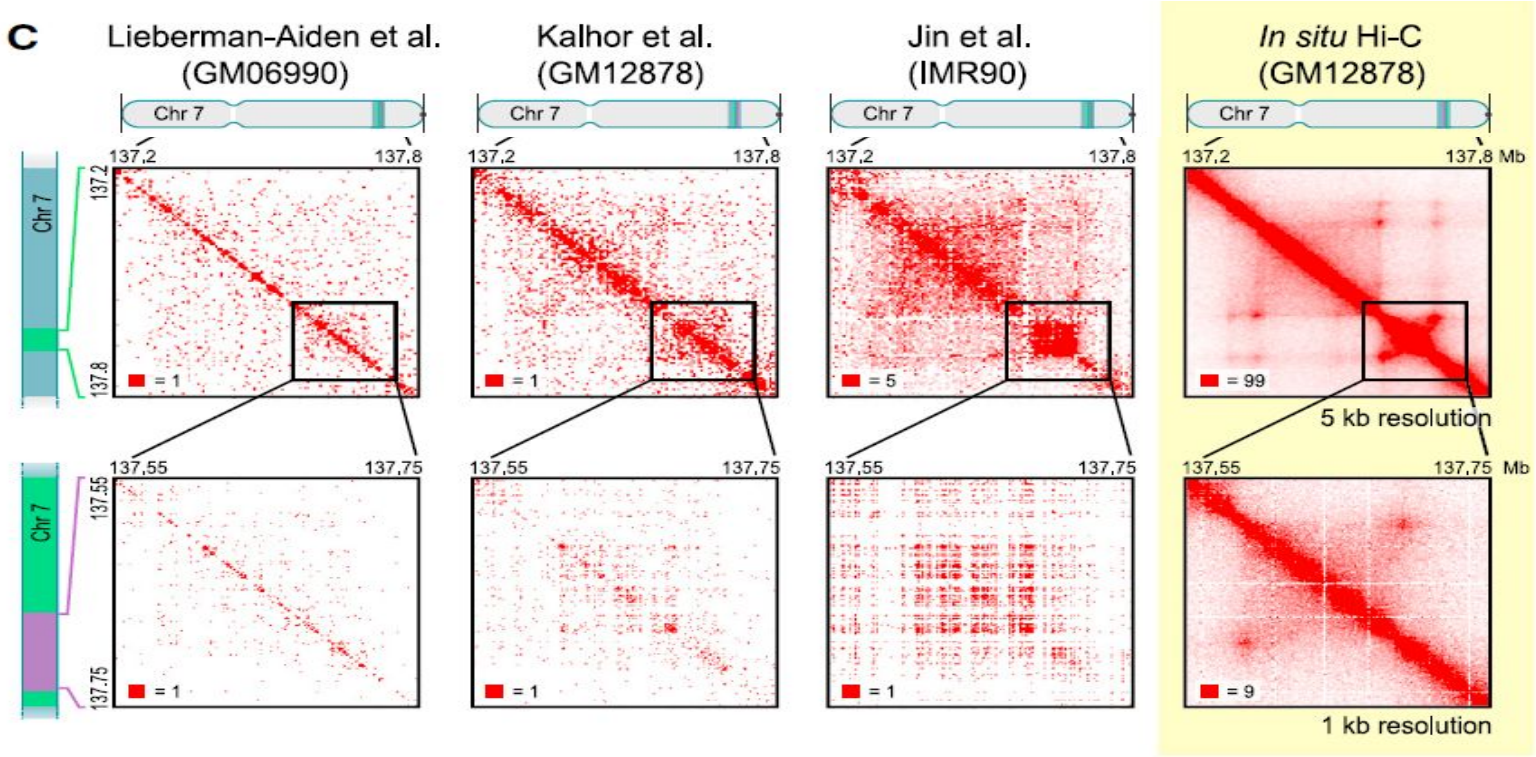
Sanborn AL, Rao SS, Huang S-CC, Durand NC, Huntley MH, Jewett AI, et al. (2015). Chromatin extrusion explains key features of loop and domain formation in wild-type and engineered genomes. Proc. Natl. Acad. Sci. U.S.A. 112: E6456–65.

How can we get rid of the high levels of background noise?



Dekker J, Marti-Renom MA, and Mirny LA. (2013). Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data.*Nat. Rev. Genet.* **14**: 390–403.

In situ Hi-C performs ligation step in intact nuclei



Adapted from Rao et al. 2014 Cell

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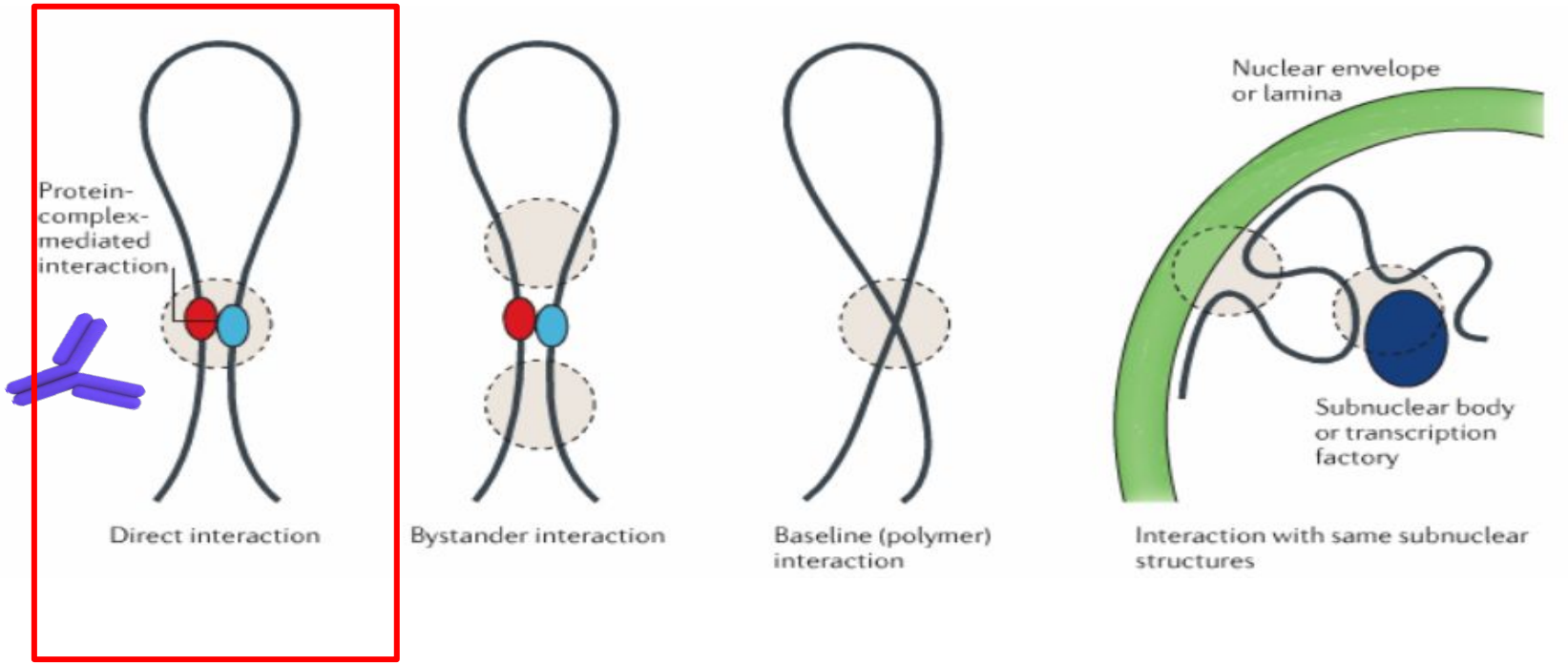
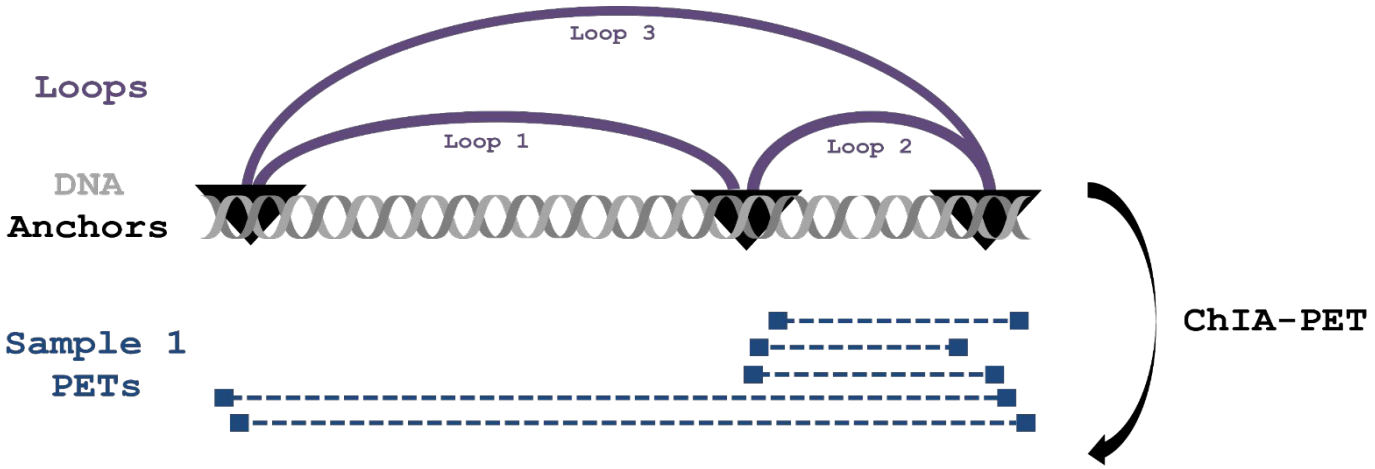


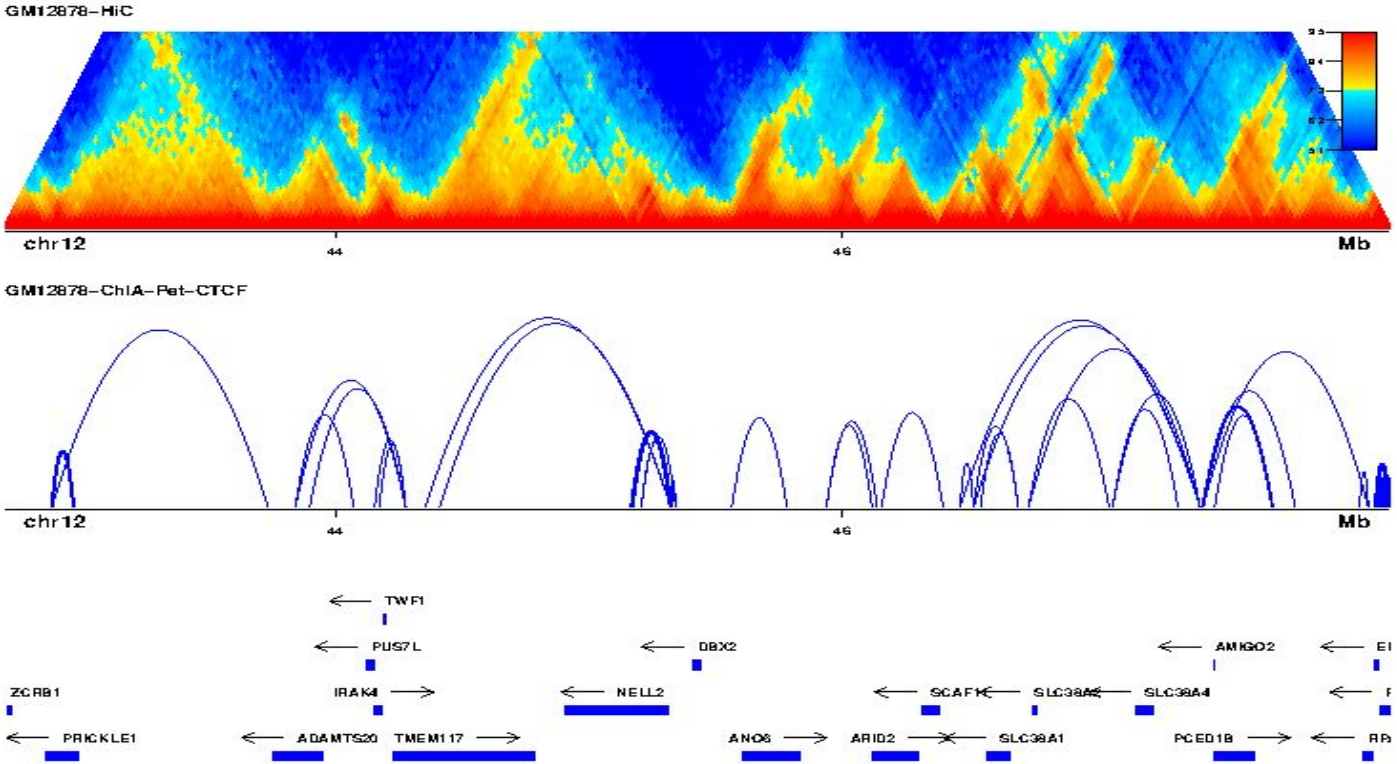
Dekker J, Marti-Renom MA, and Mirny LA. (2013). Exploring the three-dimensional organization of genomes: interpreting chromatin interaction data.*Nat. Rev. Genet.* **14**: 390–403.

Chromatin Interaction Analysis by Paired-End Tag Sequencing (ChIA-PET) to achieve enhanced specificity



From Caleb Lareau

http://dnalandscaper.aryeelab.org/



The current tools still require high amounts of input

- 6.5 billion reads from cells are golden standard for in situ HiC experiments
- 120 millions of cells would be needed for a duplicate ChIA-PET experiment
- Results in small fraction of informative reads for a given sequencing depth

To overcome these issues:

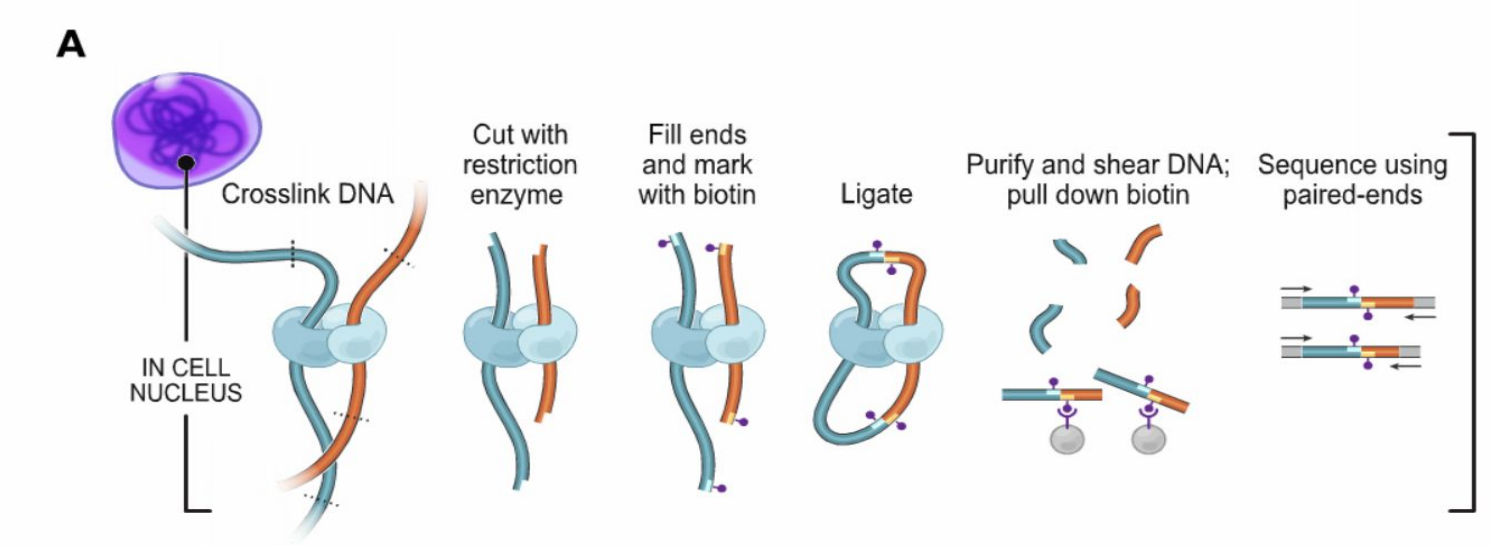
- Perform in situ DNA contacts
- Transposase mediated on-bead library construction

HiChIP

Methodology of the paper

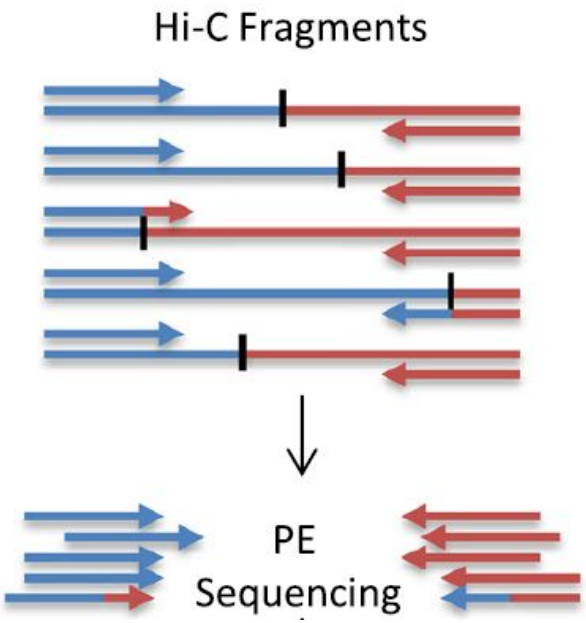
- HiChIP against Smc1a in human B lymphocyte
  and mouse embryonic stem cells
- HiC-pro pipeline to process the data

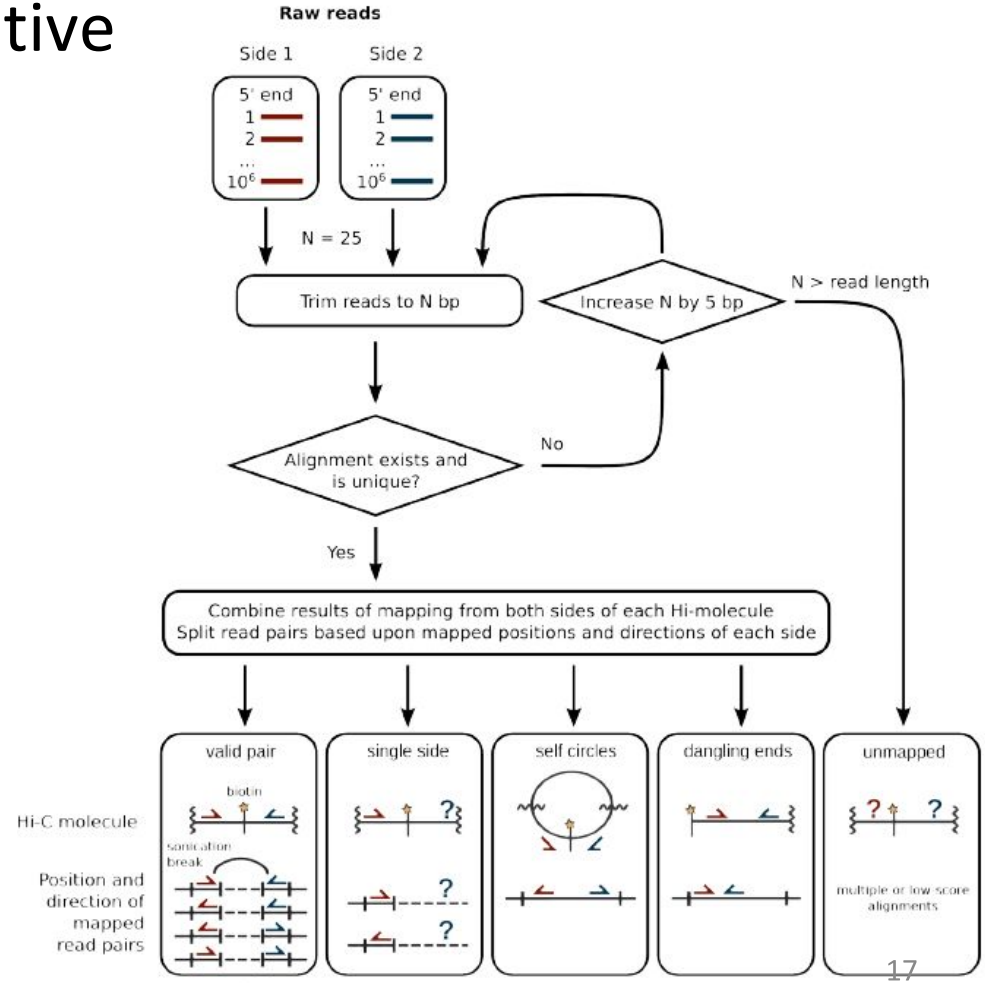
The Hi-C method was the first unbiased and genome-wide adaptation of 3C



Rao SS, Huntley MH, Durand NC, Stamenova EK, Bochkov ID, Robinson JT, *et al.* (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* **159**: 1665–80.

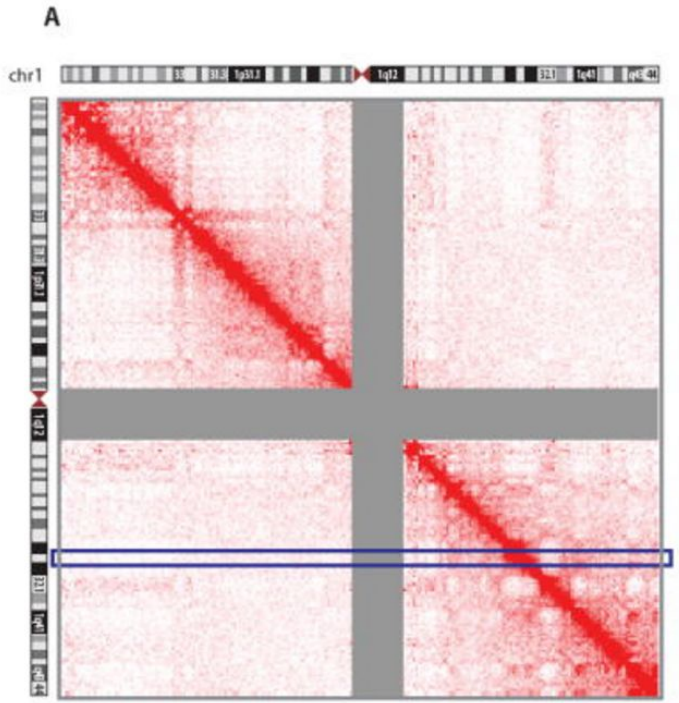
HiC-pro pipeline uses iterative correction

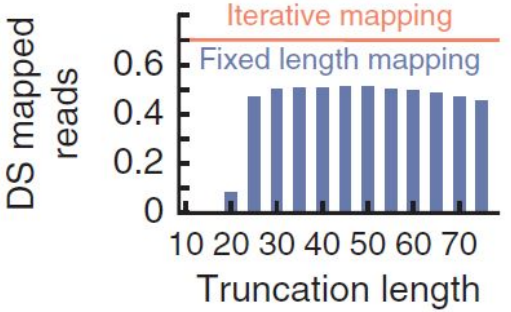


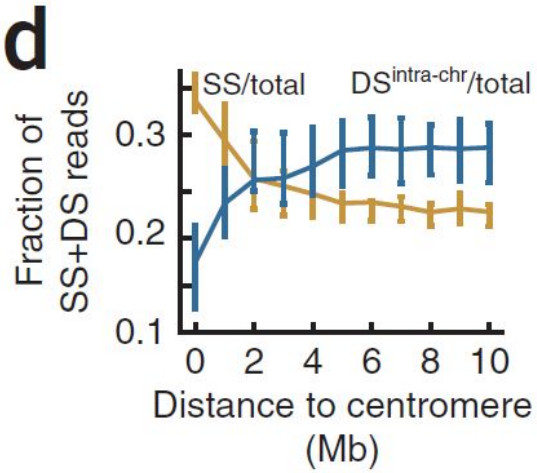


Imakaev et al. Nat. Methods (2012)
Servant et al. Genome Biology (2015)

Iterative correction achieves equal visibility

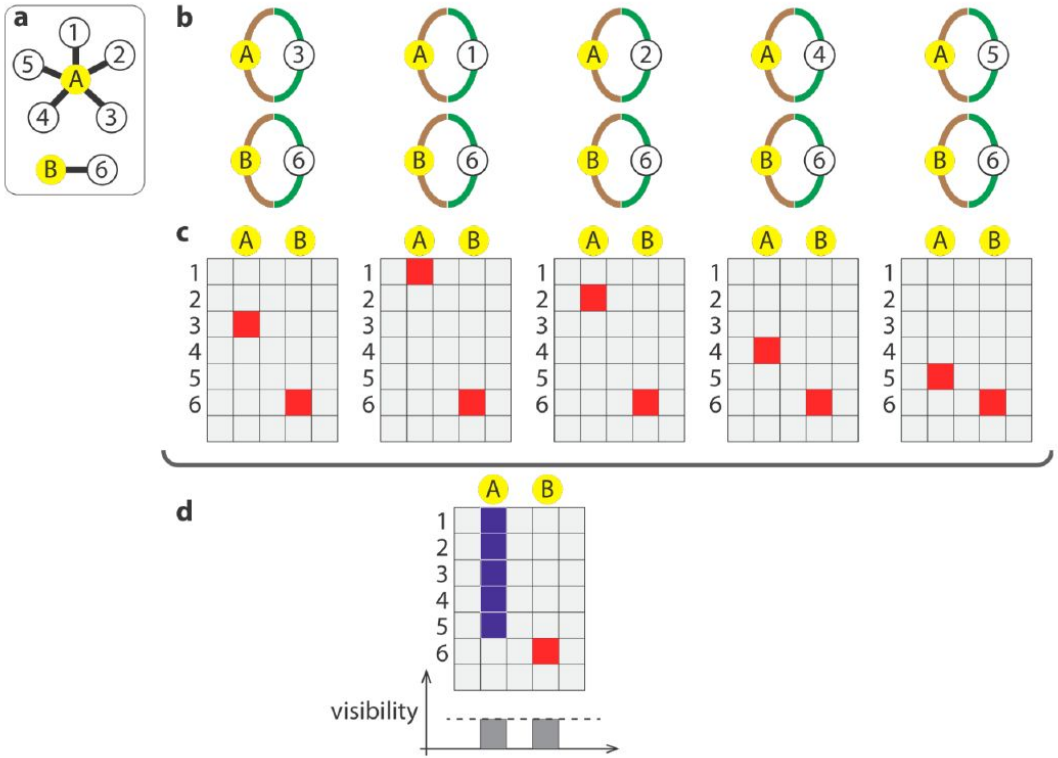


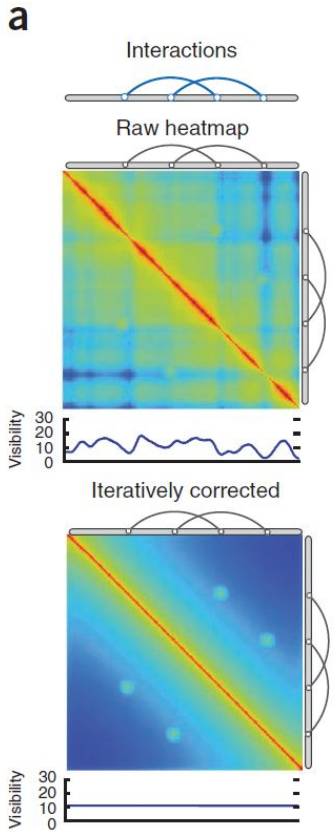




Imakaev et al. Nat. Methods (2012)
Belton et al. Methods (2012)

Equal visibility can reveal specific interactions otherwise buried





Imakaev et al. Nat. Methods (2012)

Results from the HiChIP:
Round 1: HiChIP vs ChIA-PET

HiChIP displays dramatically higher % of informative PETs

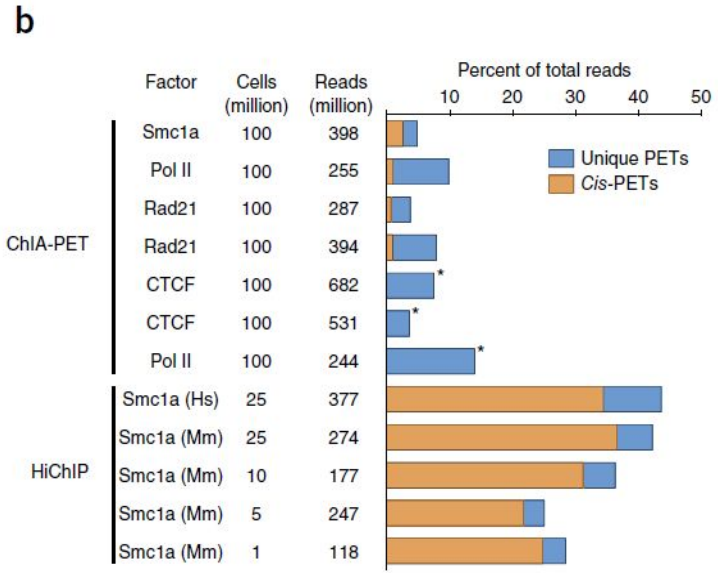
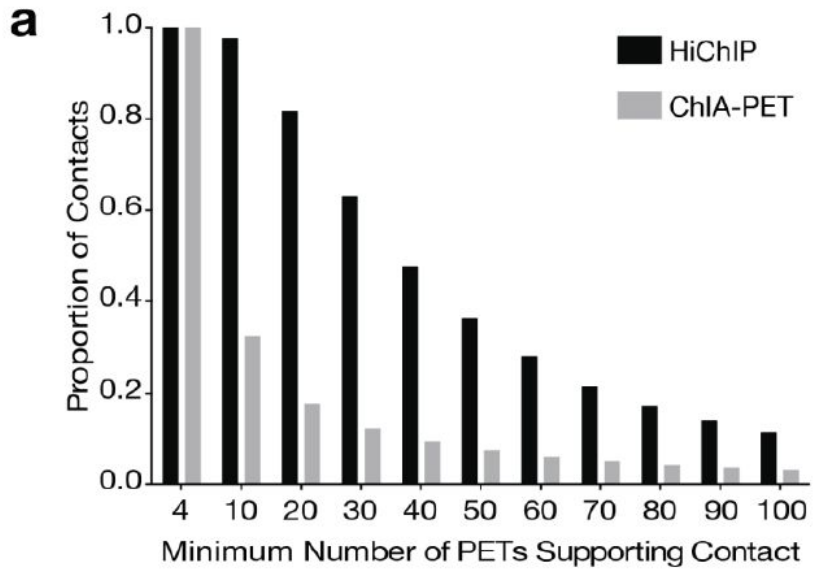
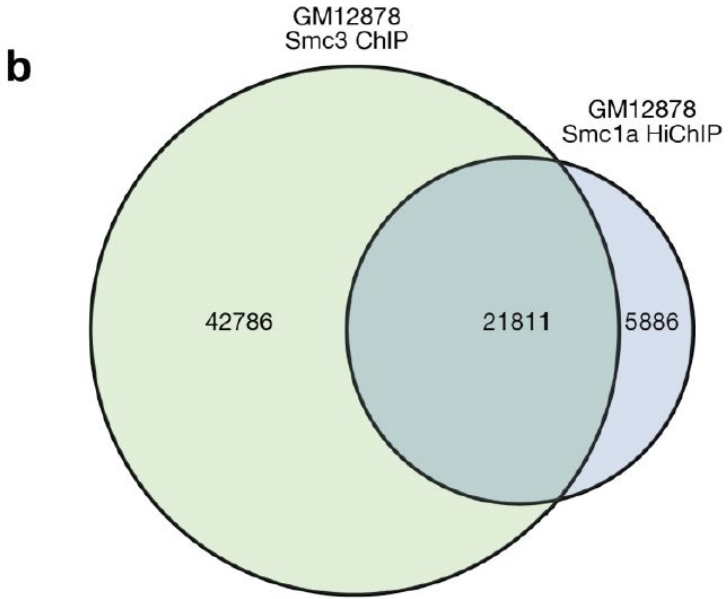


Fig 1b



Supp. Fig. 1a

Overlap of cohesin ChIP peaks from GM12878 Smc1a HiChIP and ENCODE Smc3 ChIP-seq



Supp. Fig 1b

Reproduction of a previous observation and testing lower cell inputs

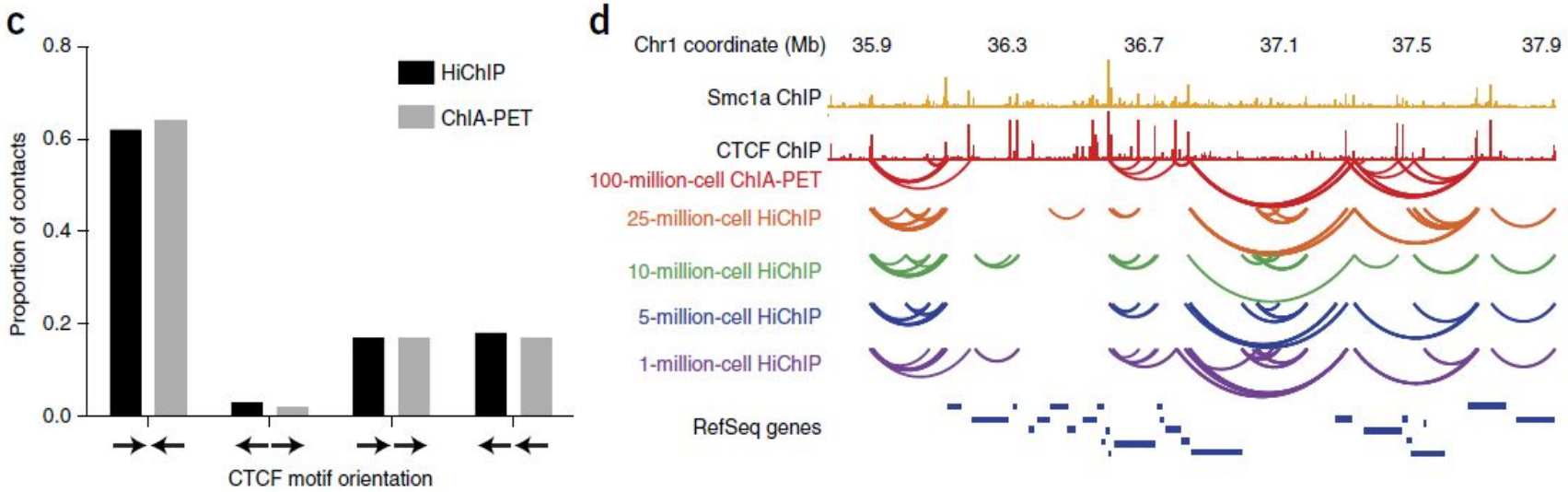
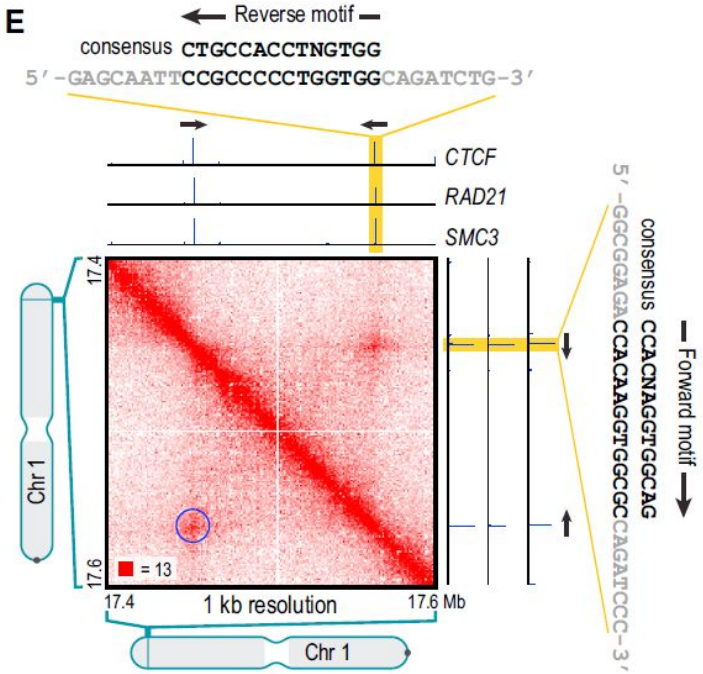
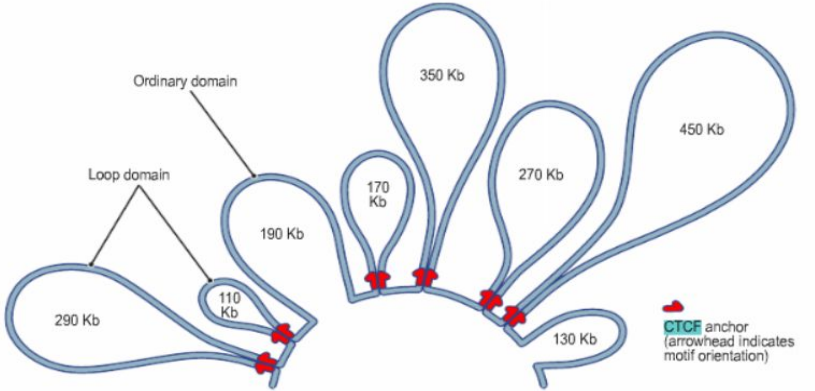


Fig 2

To be able to interpret Figure 2: Loops Are Anchored at a Pair of Convergent CTCF/RAD21/SMC3





Rao et al. Cell. 2014

Round 2: HiChIP vs in-situ HiC

HiChIP indentifies chromatin features originally found in Hi-C

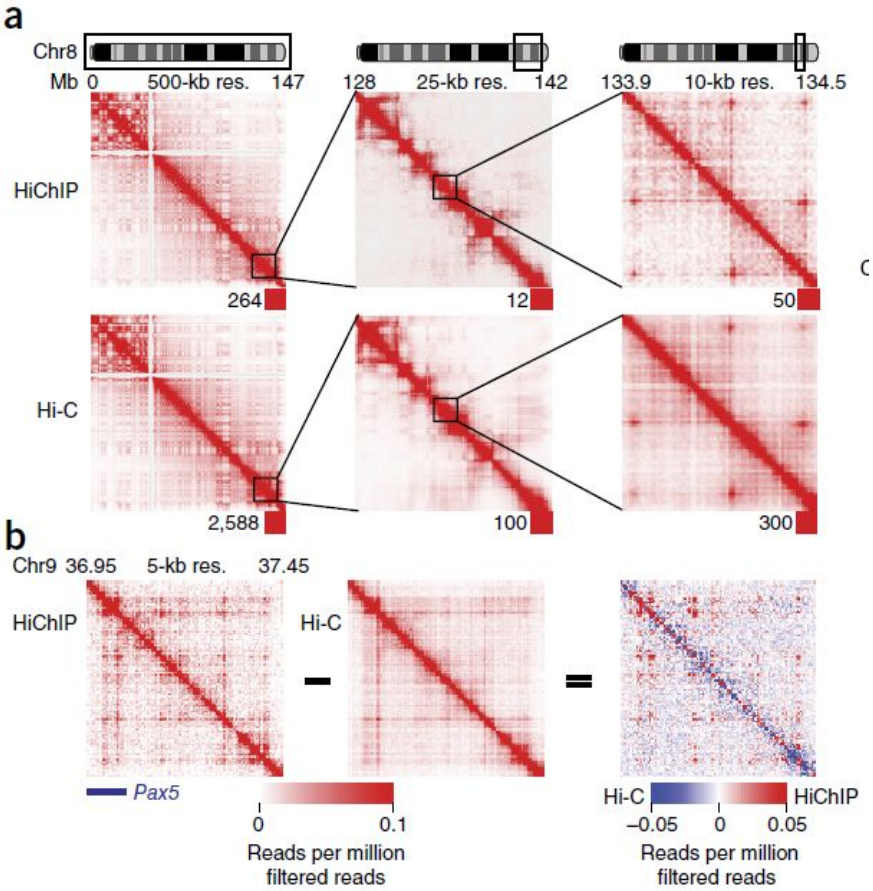
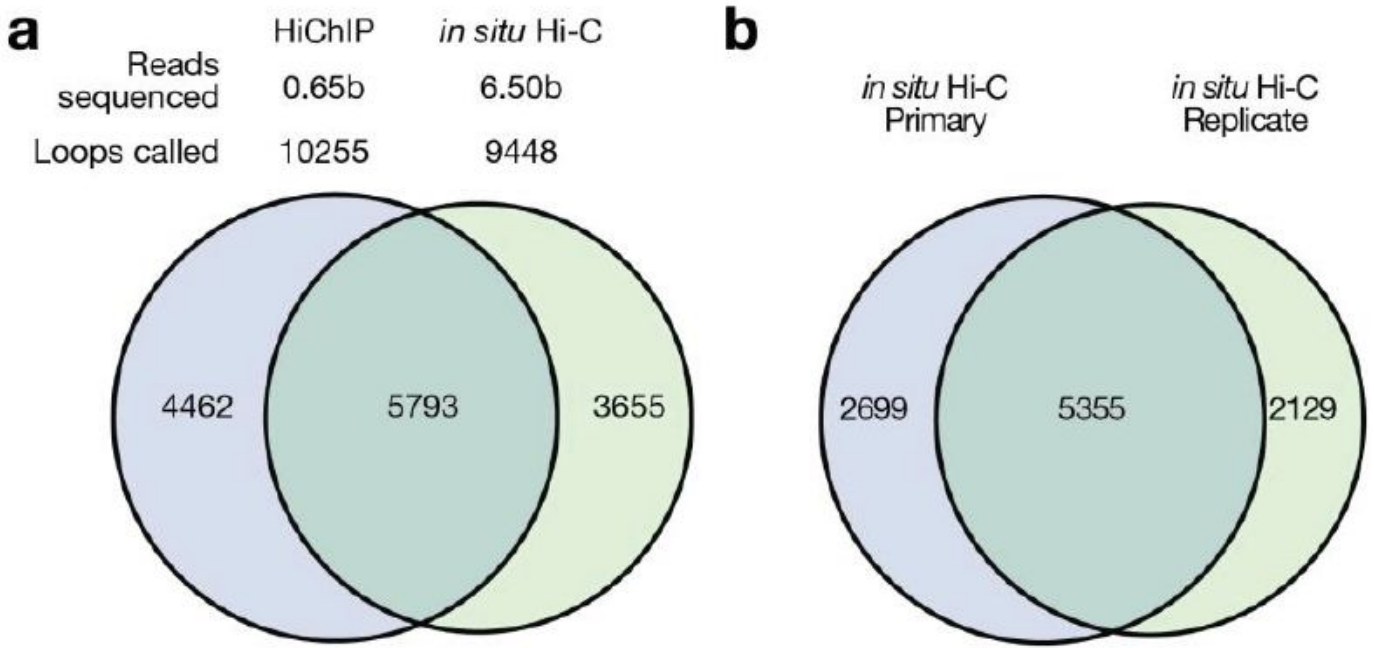


Fig 2a

HiChIP indentifies chromatin features originally found in Hi-C



Supp. Fig 2a and 2b

HiC exhibits a higher signal-to-background ratio

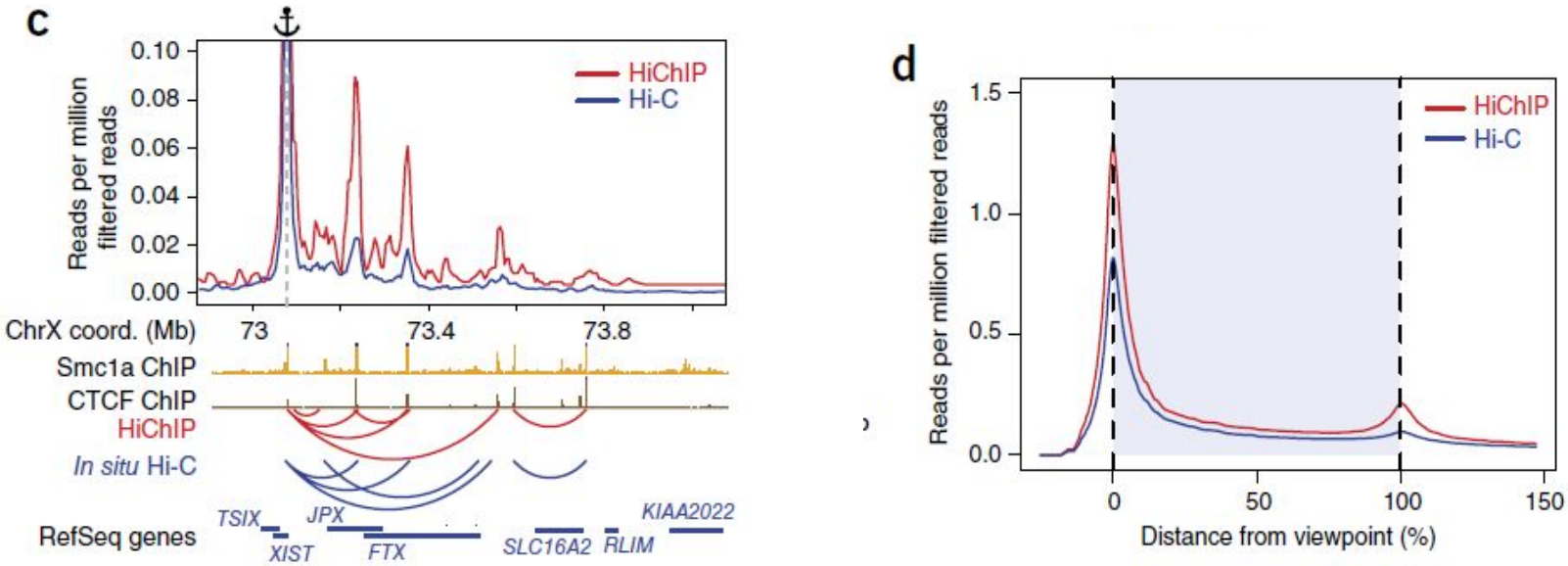


Fig 2c and 2d