

nature > scientific reports > articles > article



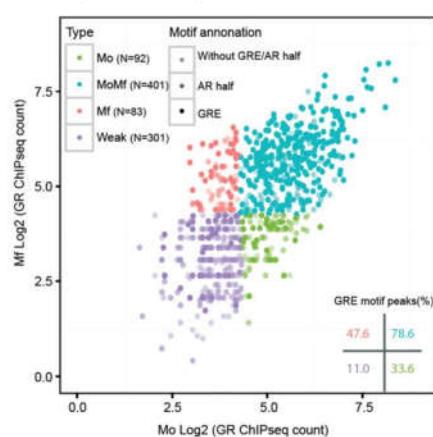
SCIENTIFIC REPORTS

Article | OPEN | Published: 26 February 2019

Extensive epigenomic integration of the glucocorticoid response in primary human monocytes and *in vitro* derived macrophages

Cheng Wang, Luca Nanni, Boris Novakovic, Wout Megchelenbrink, Tatyana Kuznetsova, Hendrik G. Stunnenberg, Stefano Ceri & Colin Logie

e GR peak steroid response element motif annotation



Motif annotation

- Without GRE/AR half
- AR half
- GRE
- Weak

GRE (de novo)

GRE (A549, Homer)

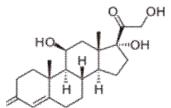
GRE (RAW, Homer)

PGR (Homer)

AR-halosite (Homer)

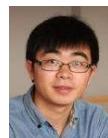
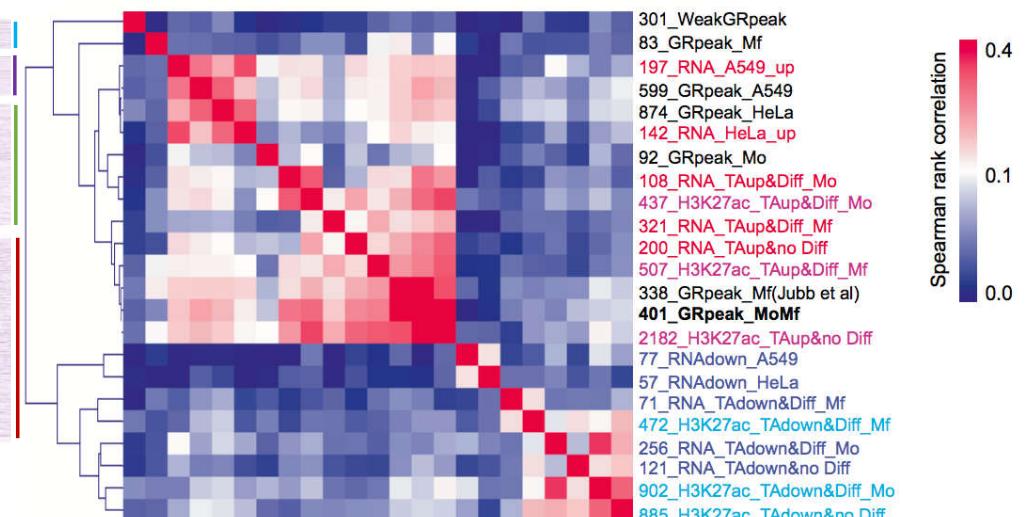
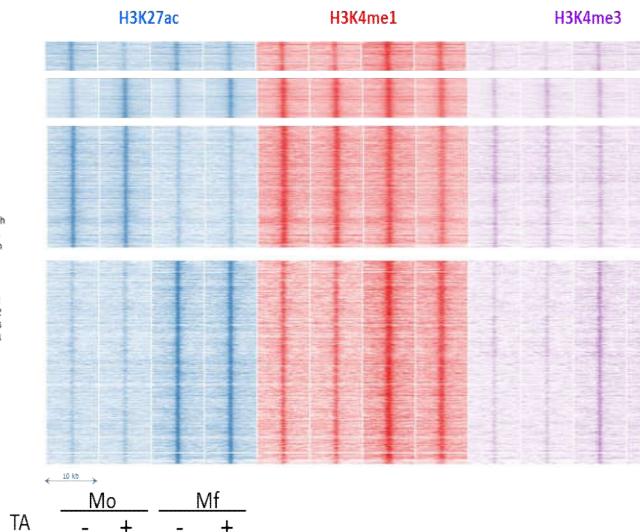
GRE motif peaks(%)

47.6 78.6
11.0 33.6



Glucocorticoid signaling

f



Cheng Wang



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Topologically associated domains (TADs) and their boundaries – CTCF site analyses

Colin Logie, PhD

Como, March 6th, 2019

What is the real world?

200 bp
200,000 bp

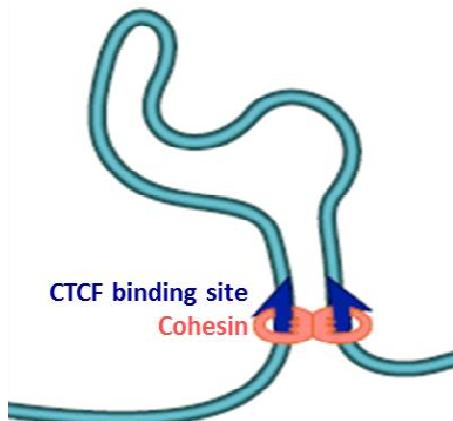
2nm wide
2nm wide

68 nm long
68 μm long

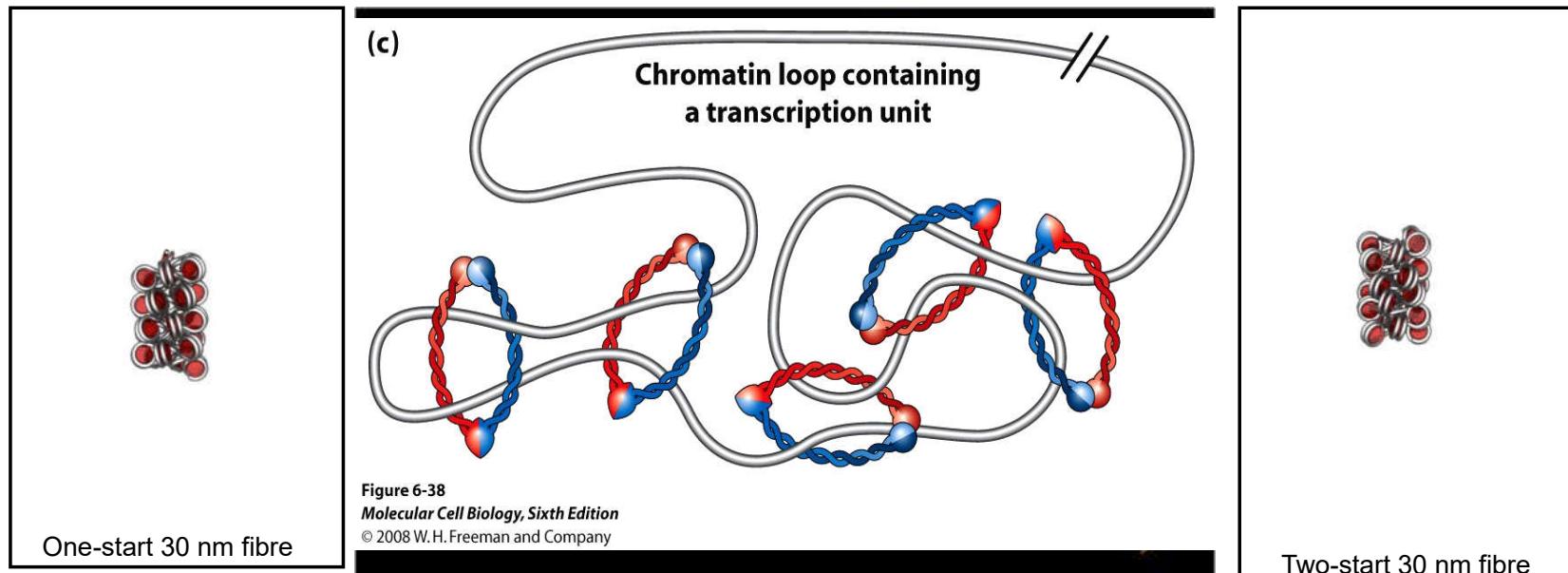
Nucleus: 5-10 μm
diameter



There is a need for DNA compaction and looping to establish chromosome territories

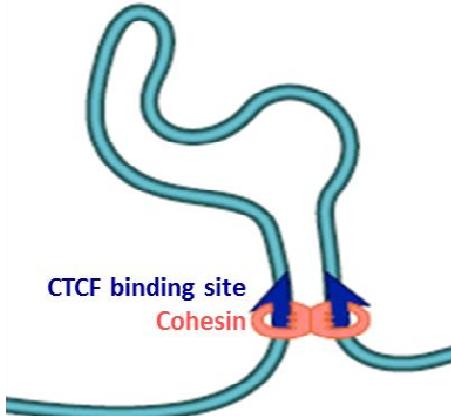


Sanborn ... Lander ... Aiden (2015)
PNAS 112, 1518552112 Figure 7E



10^3 Nucleosomes \rightarrow ~200 kb \rightarrow **6-18 μm long** poly-nucleosomal fibre

CTCF and Cohesin accumulate at the same genomic sites



Sanborn ... Lander ... Aiden (2015)
PNAS 112, 1518552112 Figure 7E

Terakawa, Science 03 Nov 2017:
Vol. 358, Issue 6363, pp. 672-676



Usually, CTCF and Cohesin occupancies do not vary much across cell types

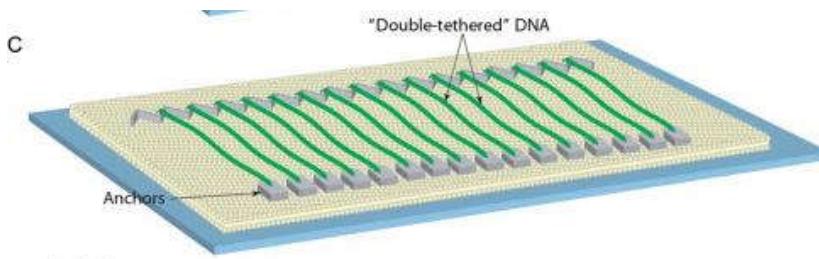


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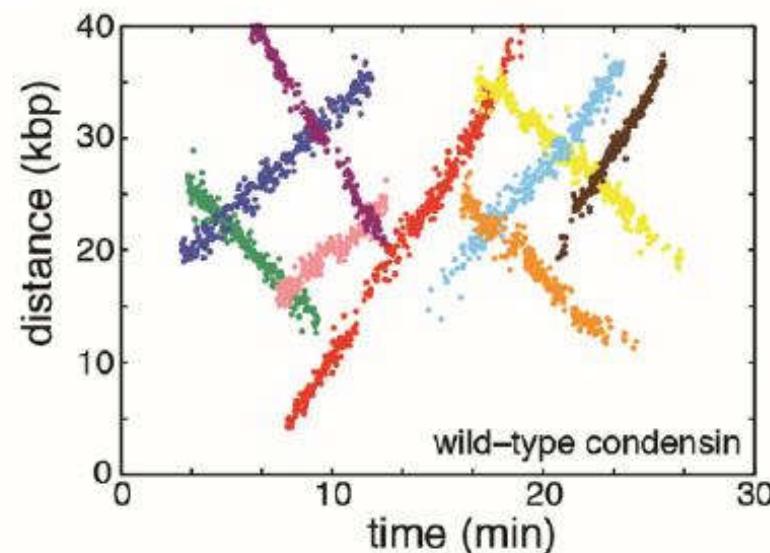
Provocative question: Is extrusion faster or slower than transcription?

03 Nov 2017

The condensin complex is a mechanochemical motor that translocates along DNA
Tsuyoshi Terakawa, Shveta Bisht, Jorine M. Eeftens, Cees Dekker, Christian H. Haering, Eric C. Greene



Methods in Cell Biology Volume 123, 2014, Pages 217-234



Science
AAAS

RNA polymerase II 200 nt/s ---- S.c. Condensin complex 30kb/10min = 50 nt/s

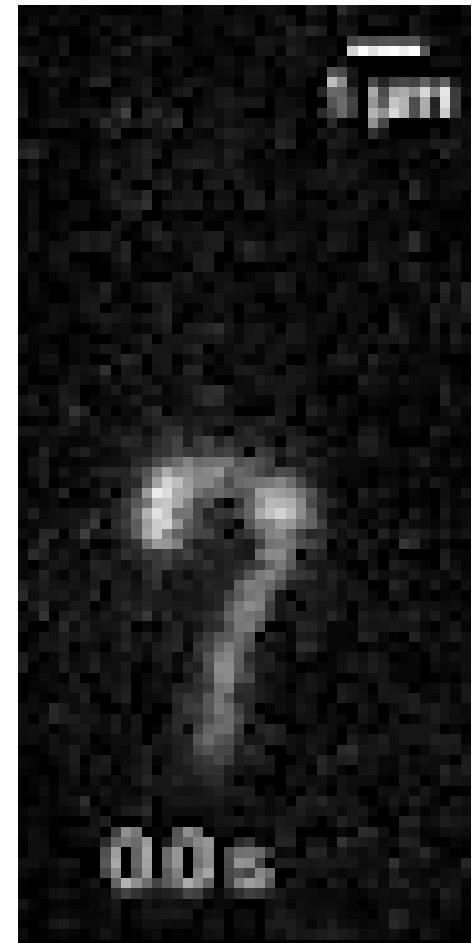
06 Apr 2018

Real-time imaging of DNA loop extrusion by condensin

Mahipal Ganji, Indra A. Shaltiel, Shveta Bisht, Eugene Kim,
Ana Kalichava, Christian H. Haering, Cees Dekker

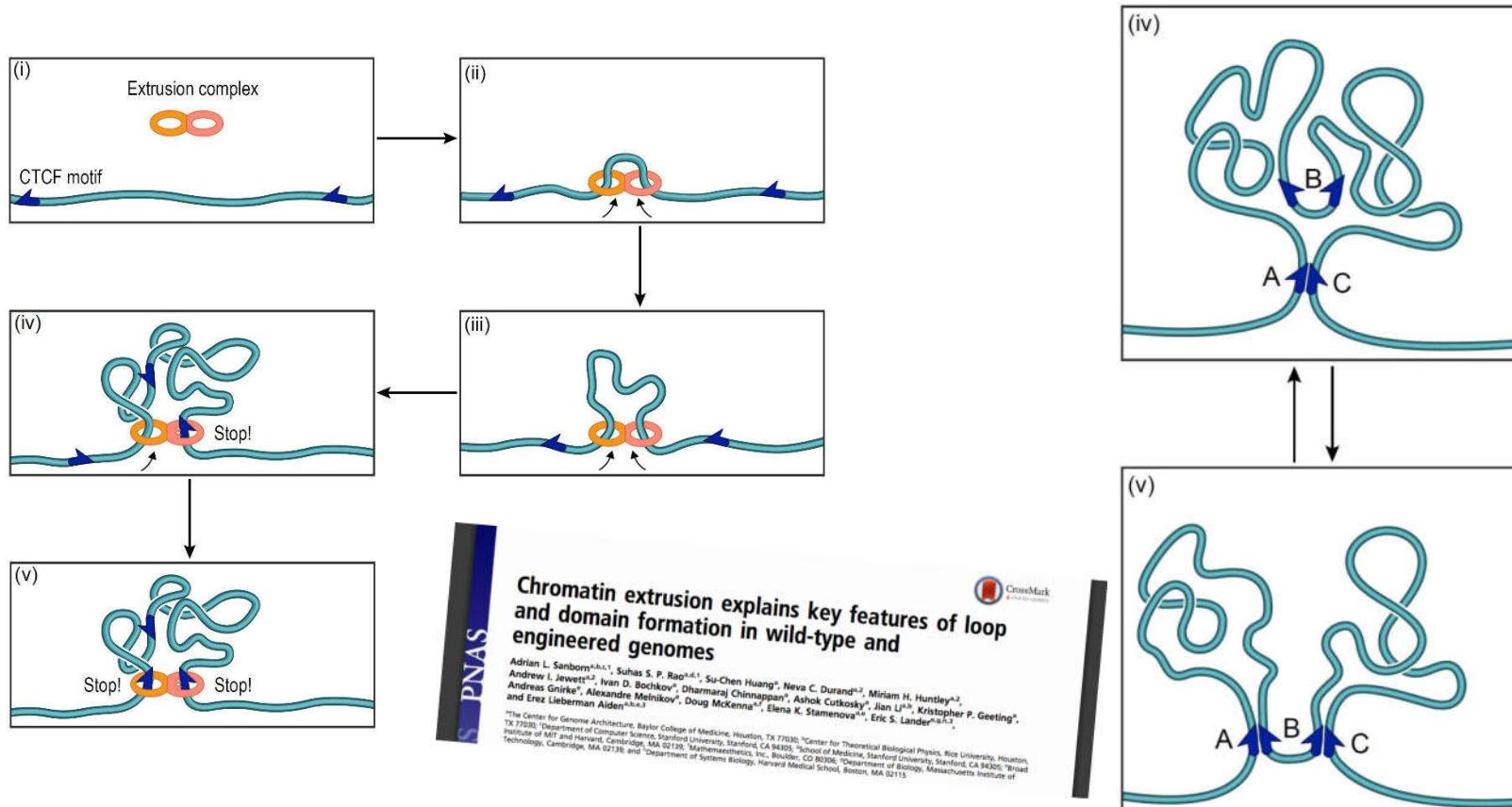
Fluorescent 16 micron (48.5 kb) DNA molecule
and a nanobead-decorated condensin complex
and ATP-Mg²⁺

Constant liquid flow to stretch DNA



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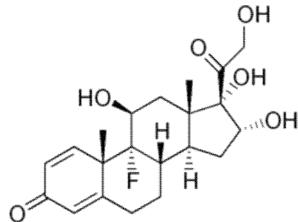
Formation of TADs -- Extrusion model



Adapted from (Sanborn et al., 2015)

HeLa cell

Triamcinolone (TA, synthetic glucocorticoid)



In the presence of steroid hormone Glucocorticoid Receptors bind to accessible DNA and recruit p300 histone acetyl transferase to an enhancer, where it acetylates H3K27. This accompanies RNA polymerase II occupancy on the target gene. Also H3K4me1 increases upon steroid treatment → more FKBP5 mRNA

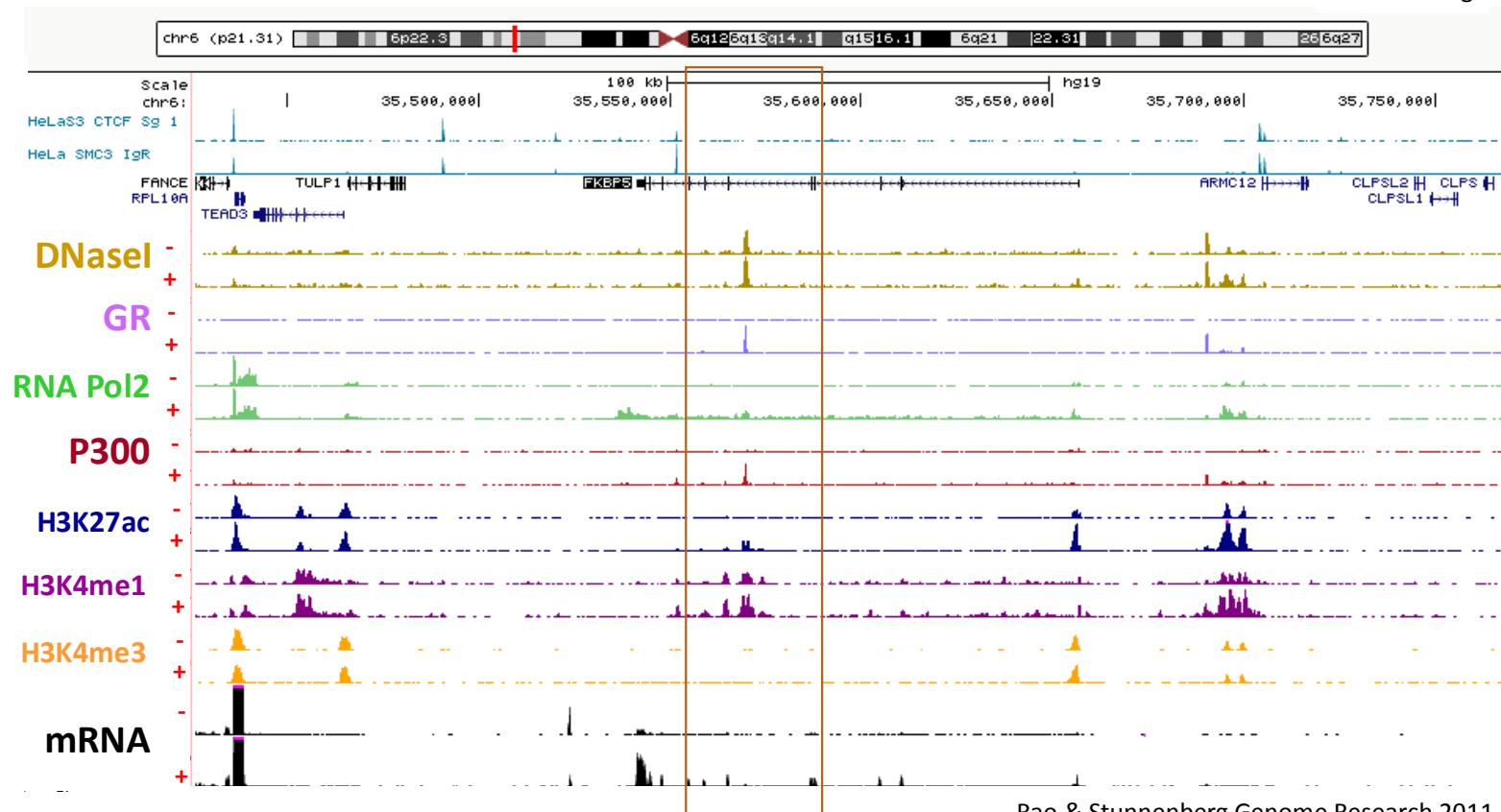
0.2 mm DNA, part of 6 cm long human chr6



Nagesha Rao

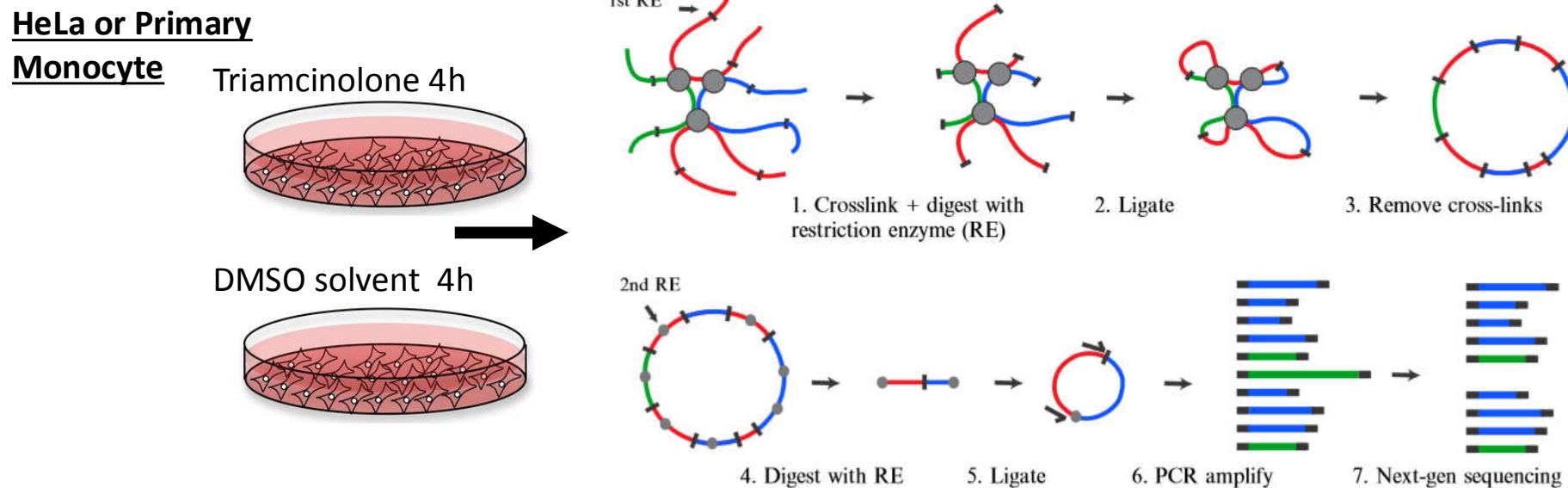


Henk Stunnenberg



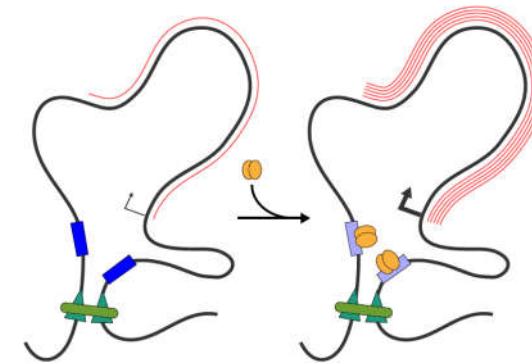
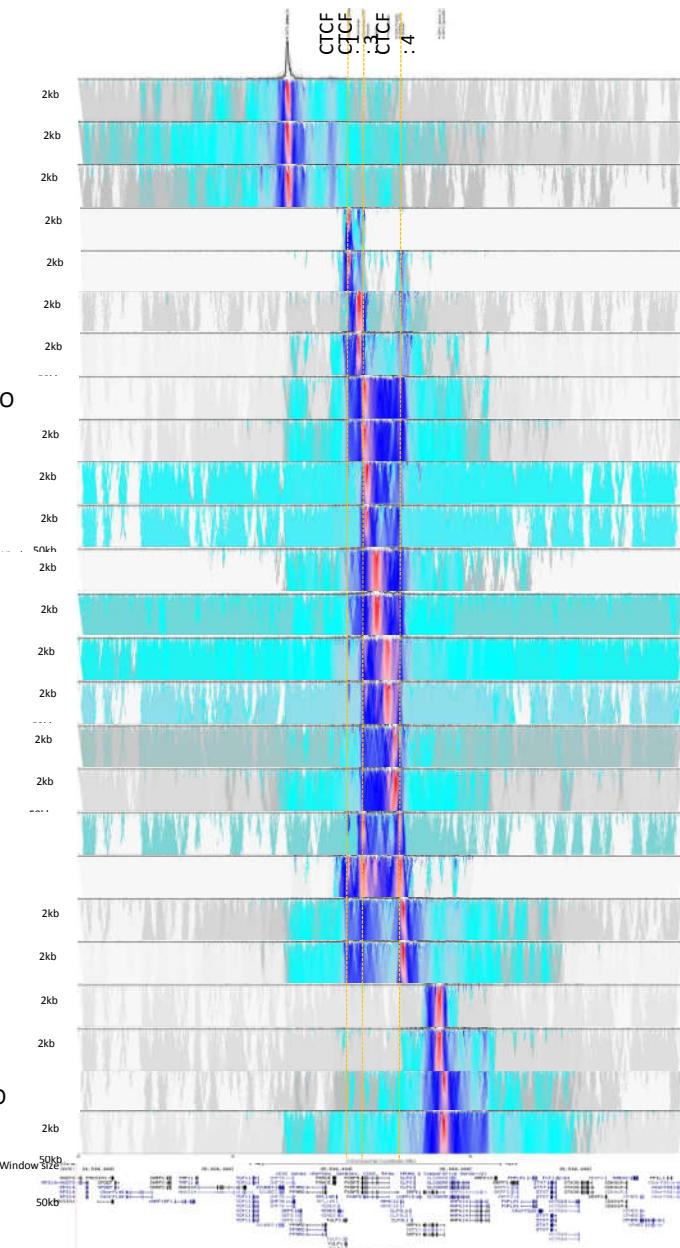
Rao & Stunnenberg Genome Research 2011

A 'one to all' chromosome conformation capture approach 4C pipeline: van de Werken Nature Methods 9, 969–972 (2012)



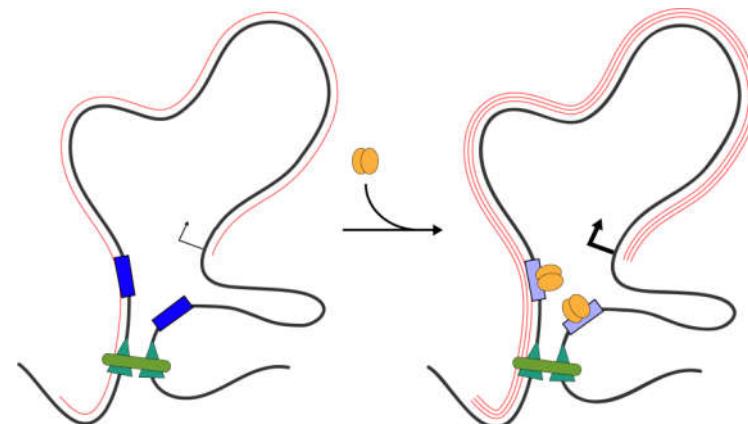
2.5 Mb chr6

ZNF76i DMSO
 ZNF76i TA
 ZNF76ii DMSO
 CTCF peak 1 DMSO
 CTCF peak 1 TA
 Downstream DMSO
 Downstream TA
 CTCF peak 6 CTCF DMSO
 CTCF peak 6 CTCF TA
 Enhancer DMSO
 Enhancer TA
 Middle DMSO
 Middle TA
 Proximal promoter
 Proximal promoter'
 -48 enhancer DMSO
 -48 enhancer TA
CTCF 7/8 DMSO
CTCF 7/8 TA
 Upstream DMSO (s9)
 Upstream TA (s9)
 SRPK1_intron2 DMSO
 SRPK1_intron2 TA
 SRPK1_promoter DMSO
 SRPK1_promoter TA



Glucocorticoids have major effects on transcription rates but little / no effect on 4C interaction frequencies

Except... for the transcribed-through CTCF site

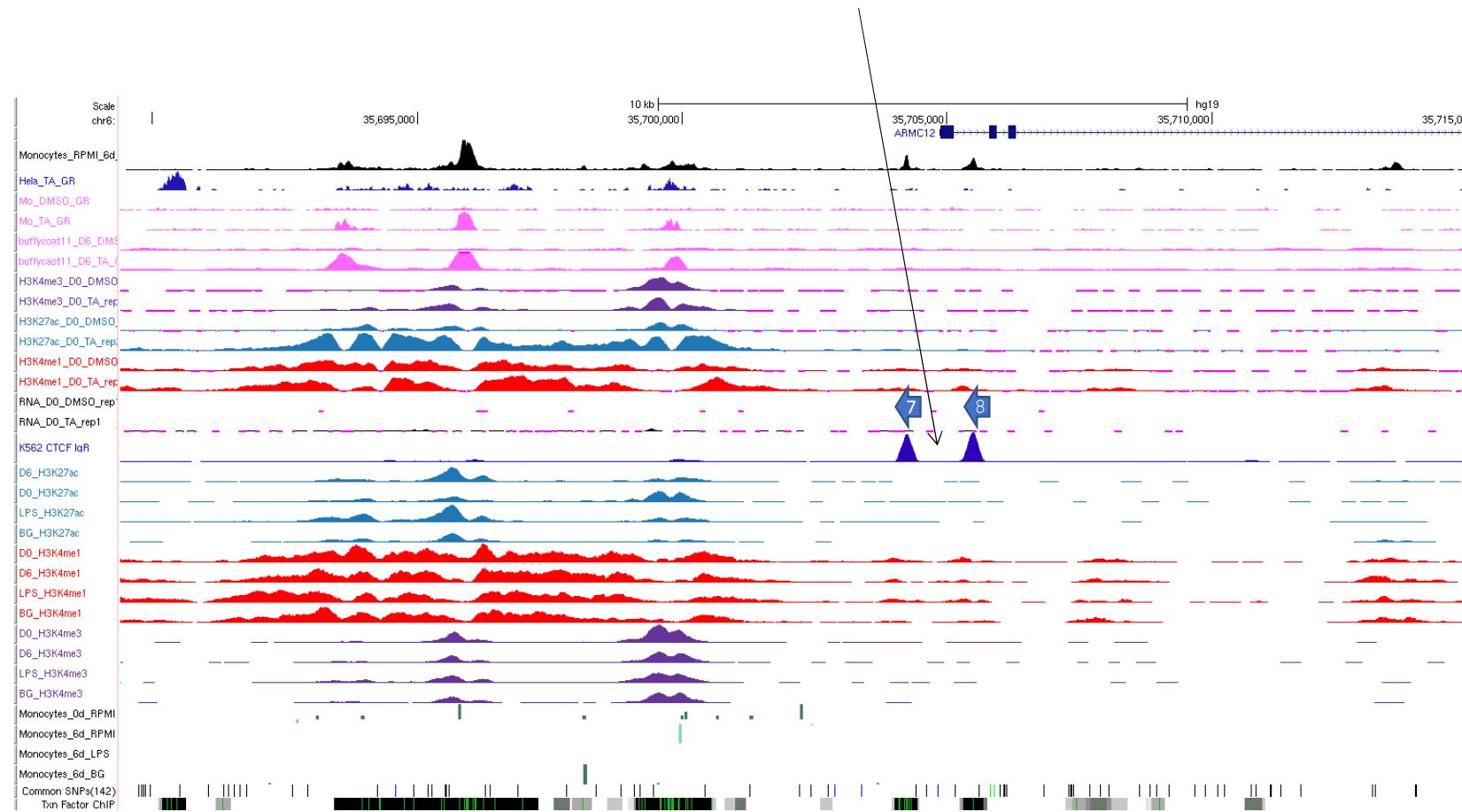


Primary monocytes

4C viewpoint between CTCF 7 and 8, which are separated by ~1000 bp



Freek Manders
Currently Utrecht
Maxima Centre



Cheng Wang



Institute for Molecular Life Sciences
Radboudumc

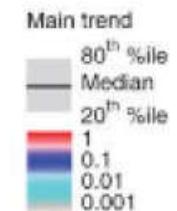
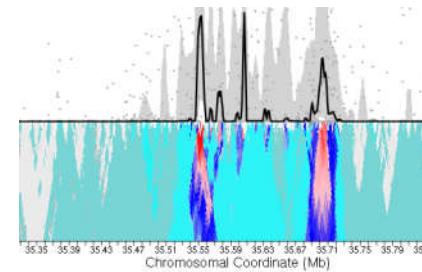
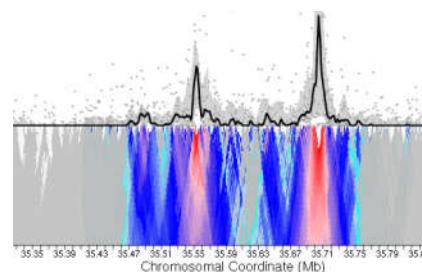
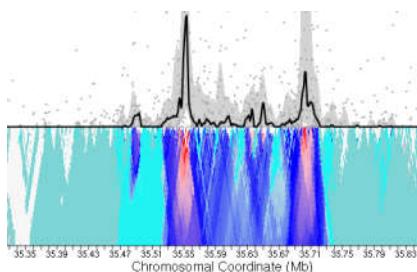
4C viewpoint between CTCF 7 and 8, which are separated by ~1000 bp

CTCF/Cohesin sites 4 5 6

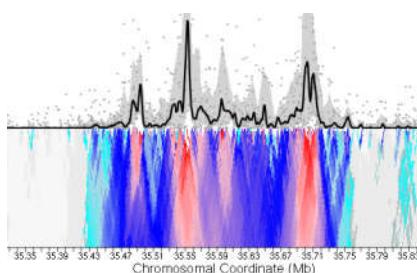


Freek Manders
Currently Utrecht
Maxima Centre
Utrecht

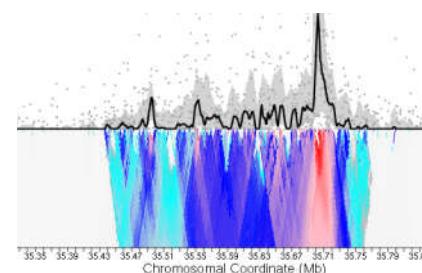
Lower transcription -- No steroid



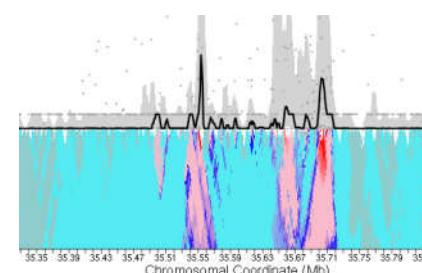
50x higher transcription -- Triamcinolone (100 nM)



HeLa cancer cell



THP1 cancer cell



Primary human blood monocyte

Cheng Wang + Freek Manders + Roland Oellers + Roel Oldenkamp

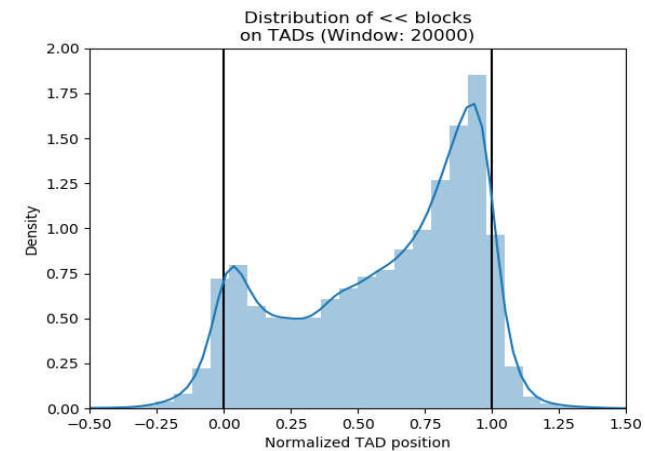
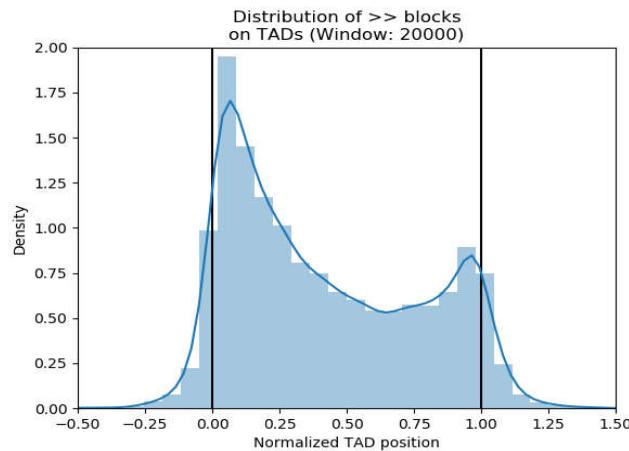
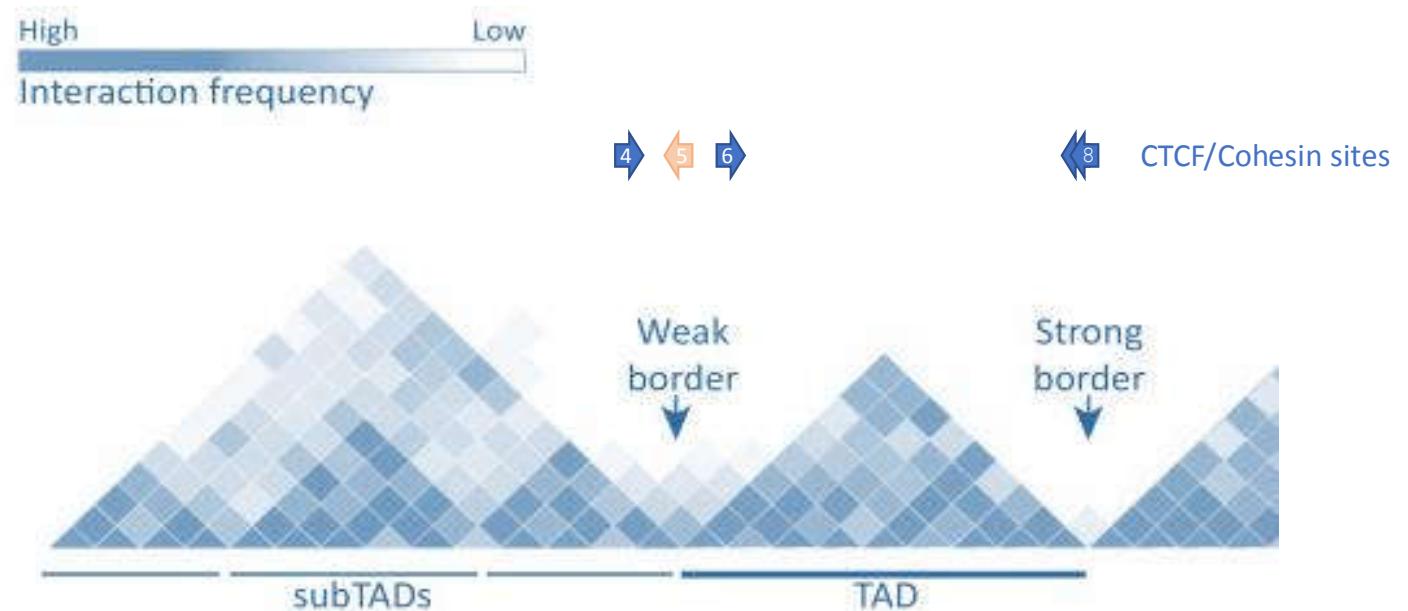
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Closely spaced CTCF

>>

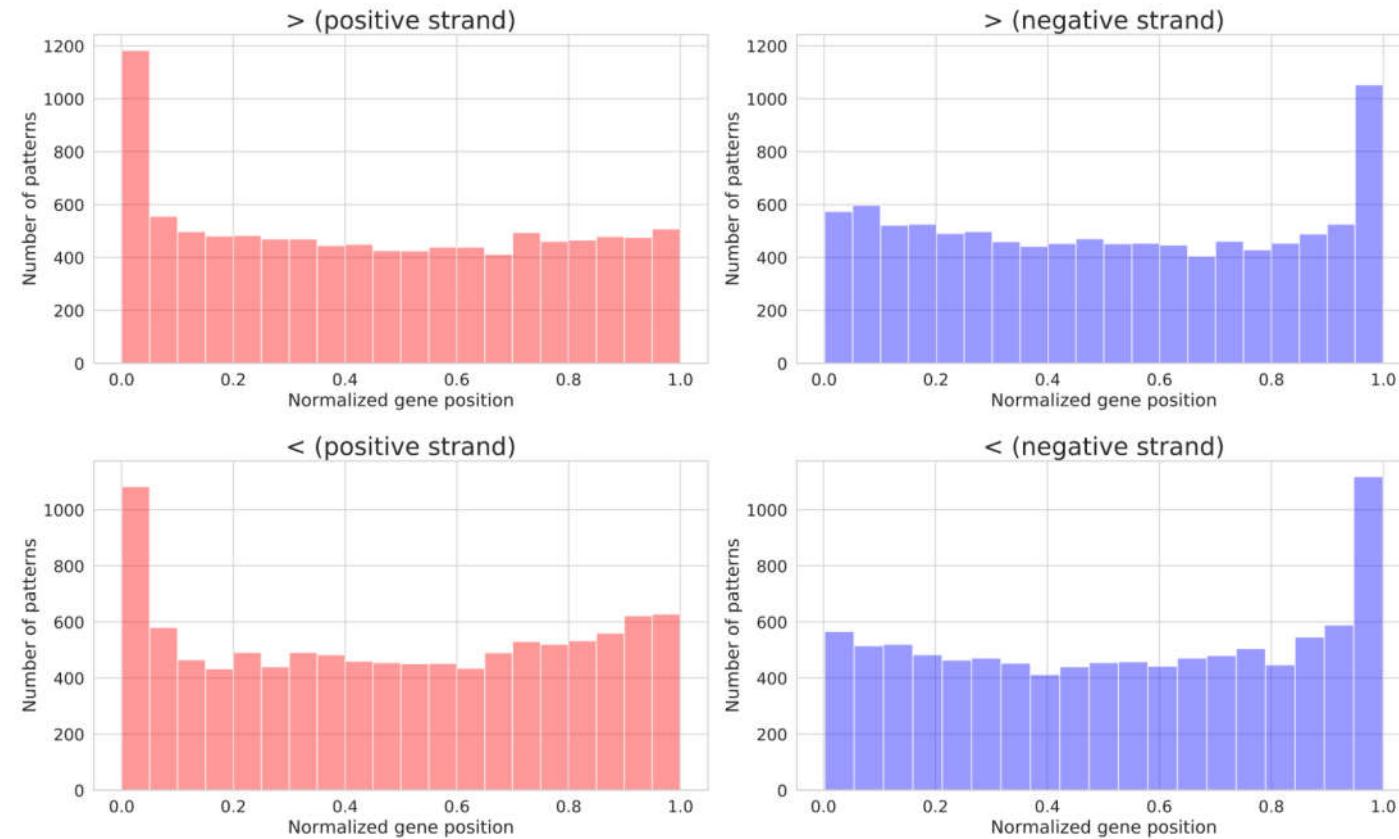
combinations
are strong TAD
borders

Weak borders have a
'back-up' CTCF at
some distance

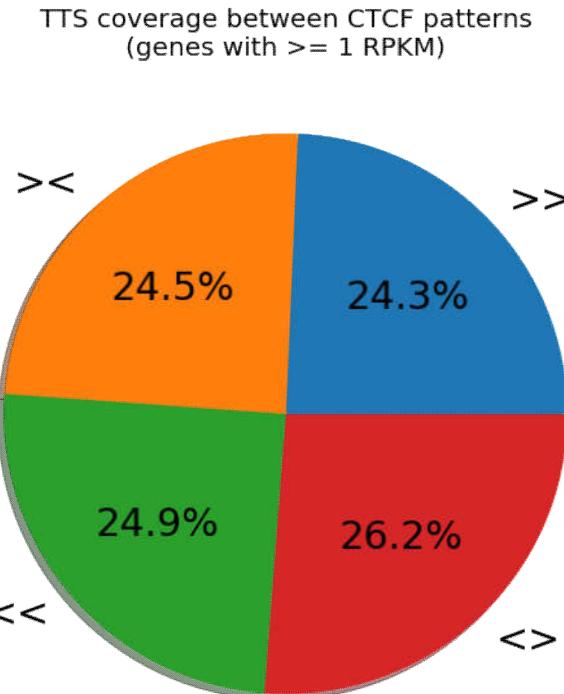
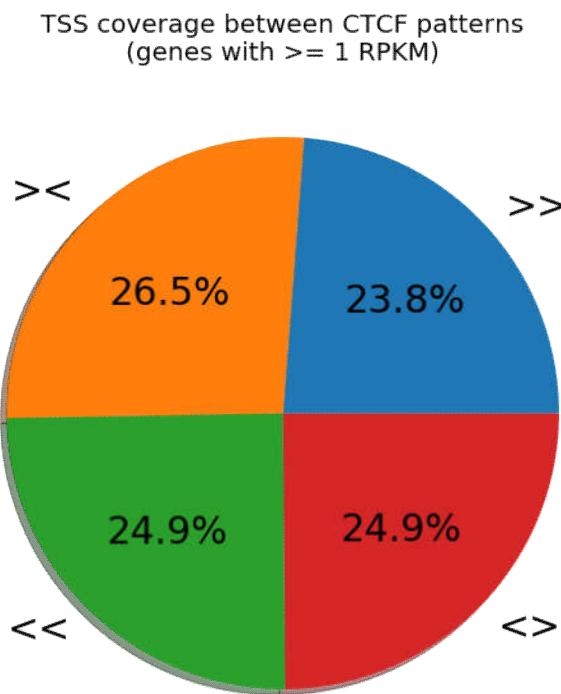


Cheng Wang

**CTCF sites in genes: Both orientations are found
More CTCF are found close to the beginning (5') of genes**



CTCF bounded DNA: gene transcription start and termination site are neutral with respect to >>, <> and >< delimited DNA intervals



Di-plets of CTCF

Same (2/4)

>>

<<

Convergent (1/4)

><

Divergent (1/4)

<>

Seven human blood cell types display rather similar TADs

200 bp	2nm wide	68 nm long	Nucleus: 5-10 μm diameter
200,000 bp	2nm wide	68 μm long	

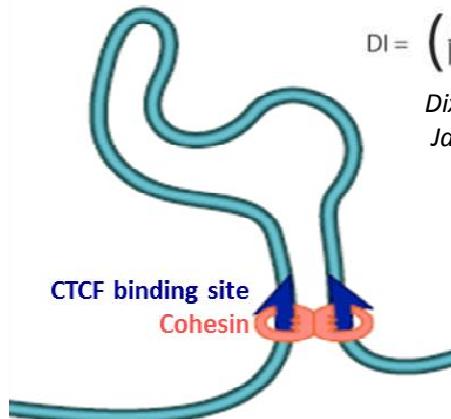


The diagram illustrates the hierarchical organization of chromatin within a cell nucleus. At the smallest scale, DNA is wrapped around nucleosomes, represented by small yellow spheres. These nucleosomes are organized into larger structures called nucleoplasmin units, shown as clusters of colored shapes (red, green, blue, purple). These units further assemble into chromatin loops, depicted as larger, more complex shapes. The entire chromatin network is contained within a nuclear envelope, represented by a double membrane boundary.

TADs determined by Blueprint

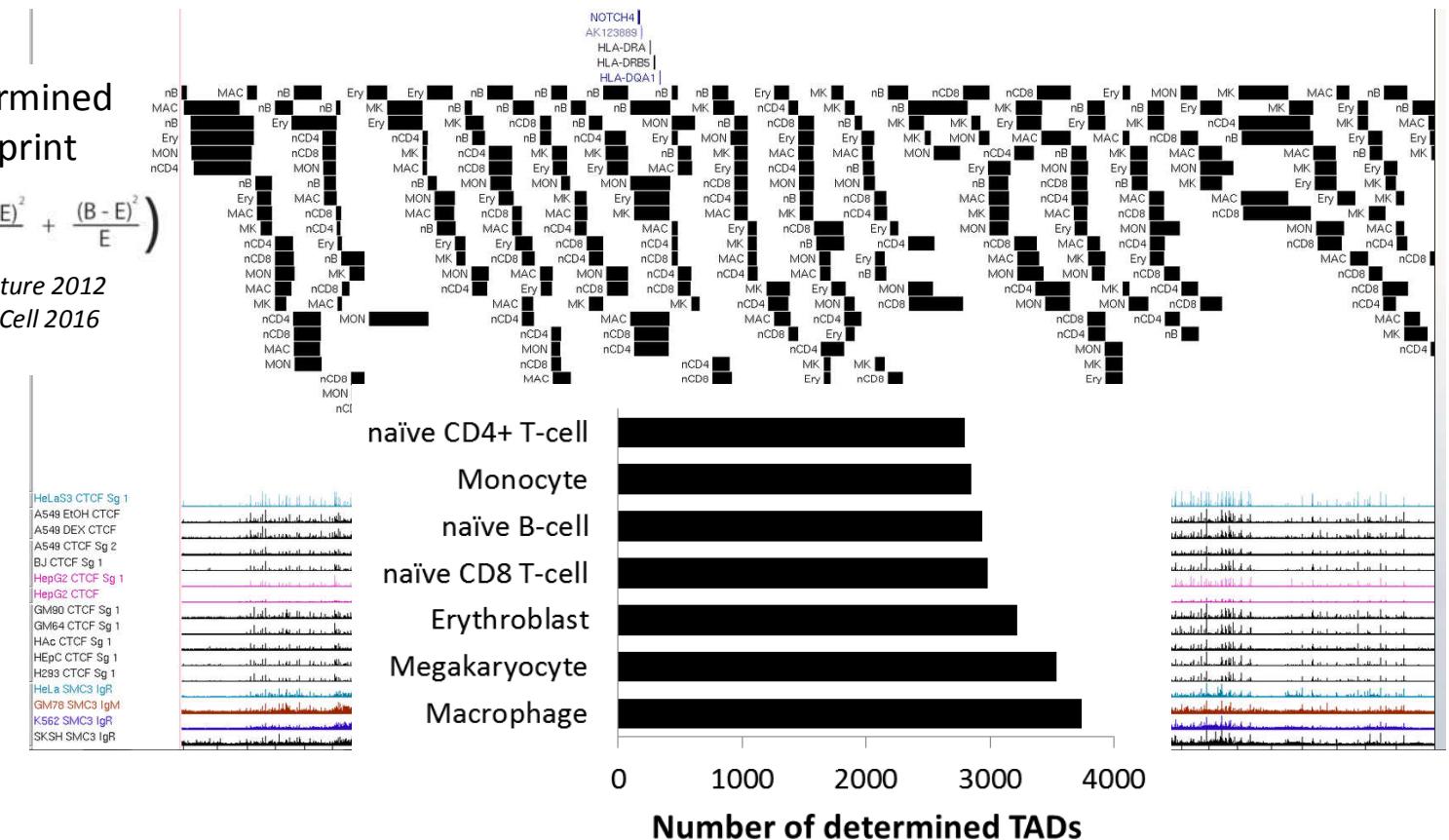
$$DI = \left(\frac{B - A}{|B - A|} \right) \left(\frac{(A - E)^2}{E} + \frac{(B - E)^2}{E} \right)$$

Dixon et al Nature 2012
Javierre et al Cell 2016

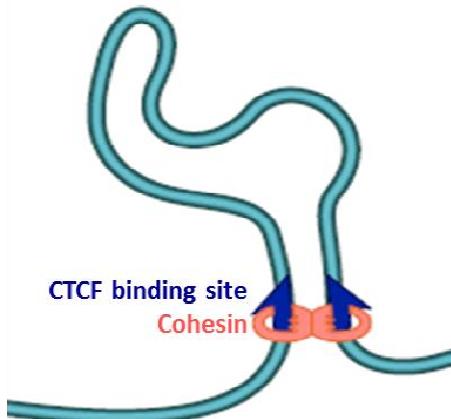


Sanborn ... Lander ... Aiden (2015)
PNAS 112, 1518552112 Figure 7E

Terakawa, Science 03 Nov 2017:
Vol. 358, Issue 6363, pp. 672-676

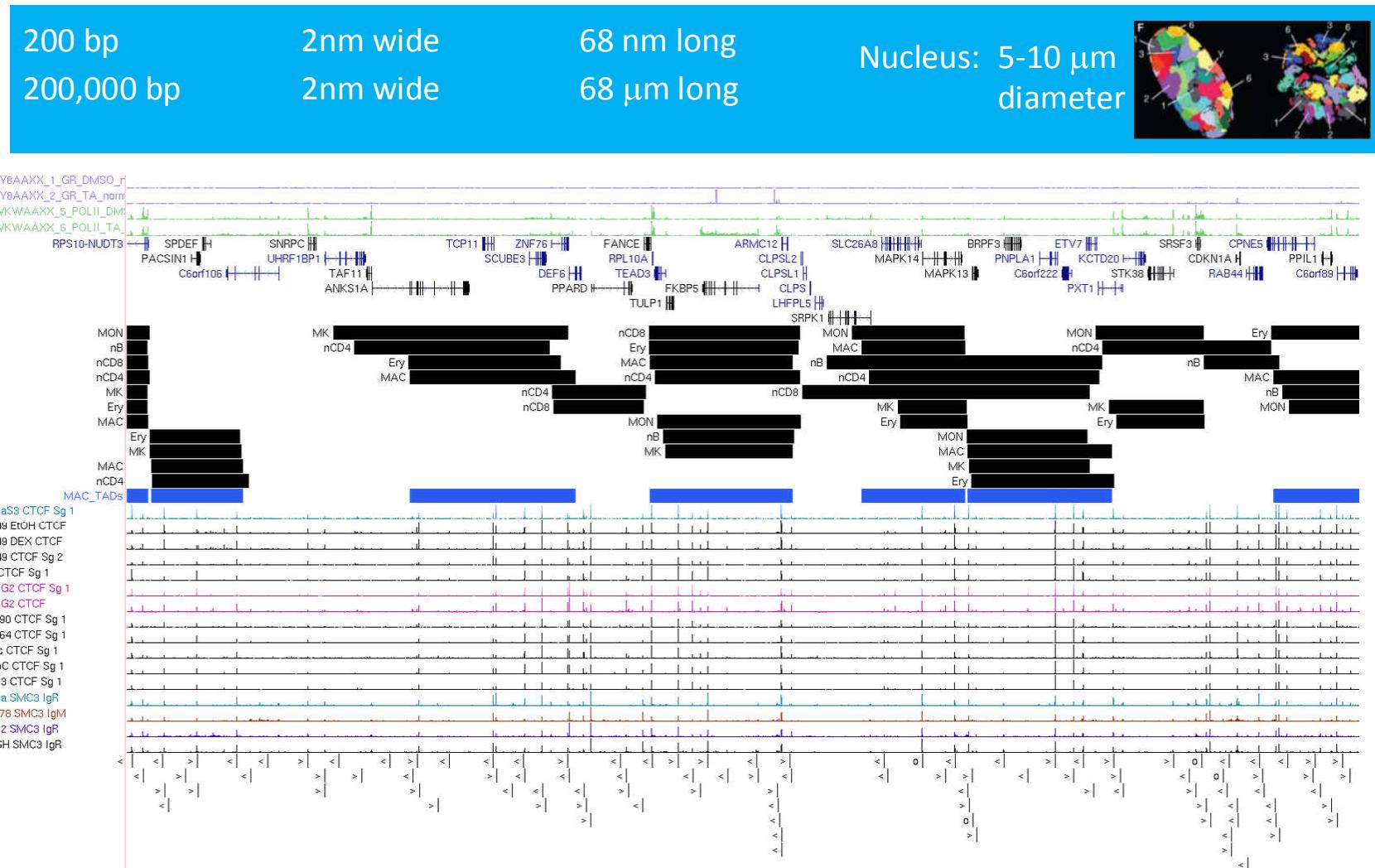


Usually, human
blood cell types
display very similar
TADs

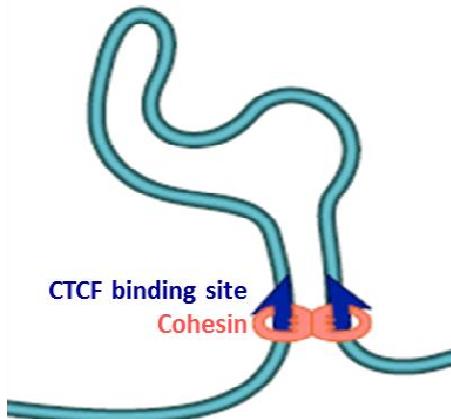


Sanborn ... Lander ... Aiden (2015)
PNAS 112, 1518552112 Figure 7E

Terakawa, Science 03 Nov 2017:
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Usually, human
blood cell types
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TADs



Sanborn ... Lander ... Aiden (2015)
PNAS 112, 1518552112 Figure 7E

Terakawa, Science 03 Nov 2017:
Vol. 358, Issue 6363, pp. 672-676

200 bp

200,000 bp

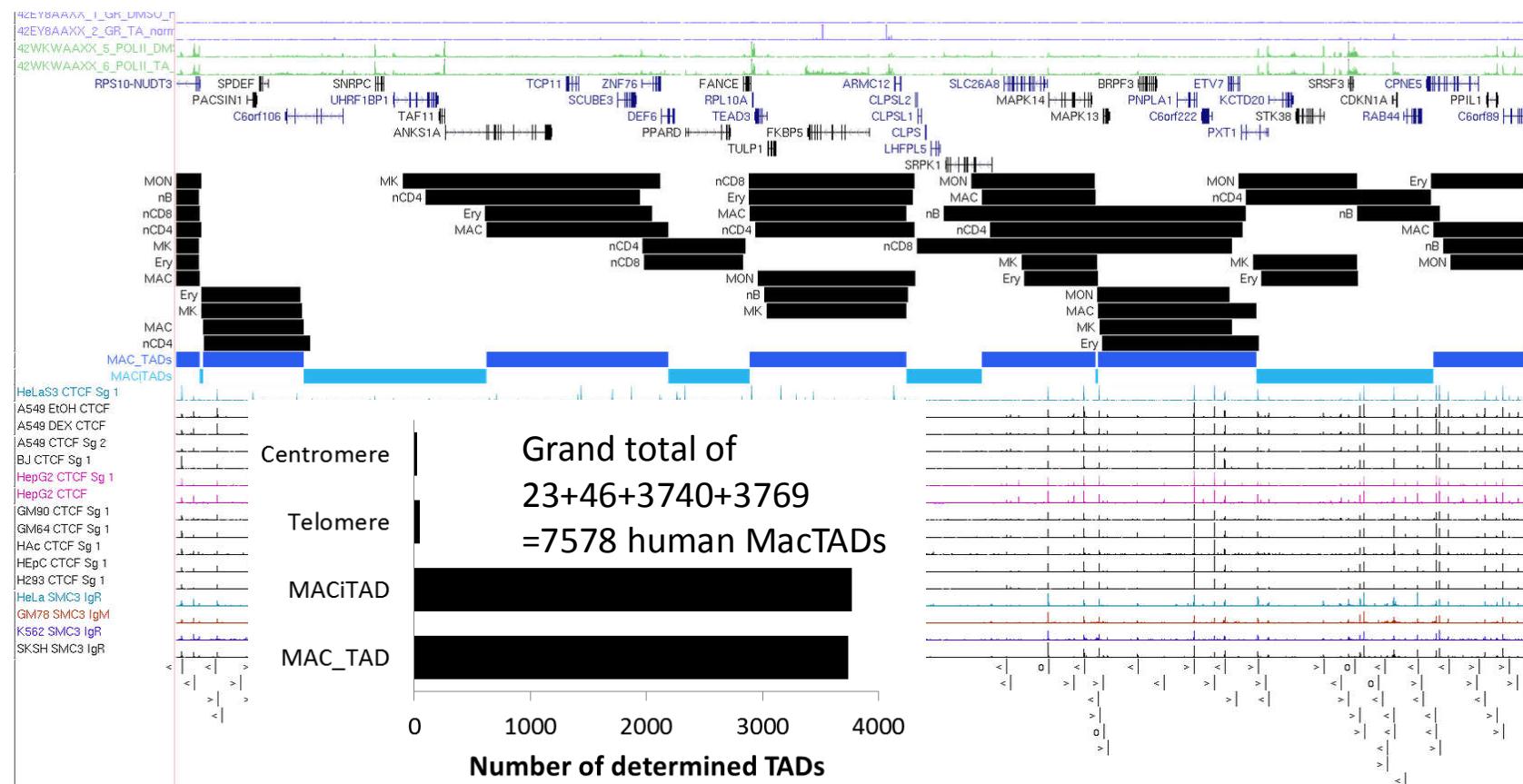
2nm wide

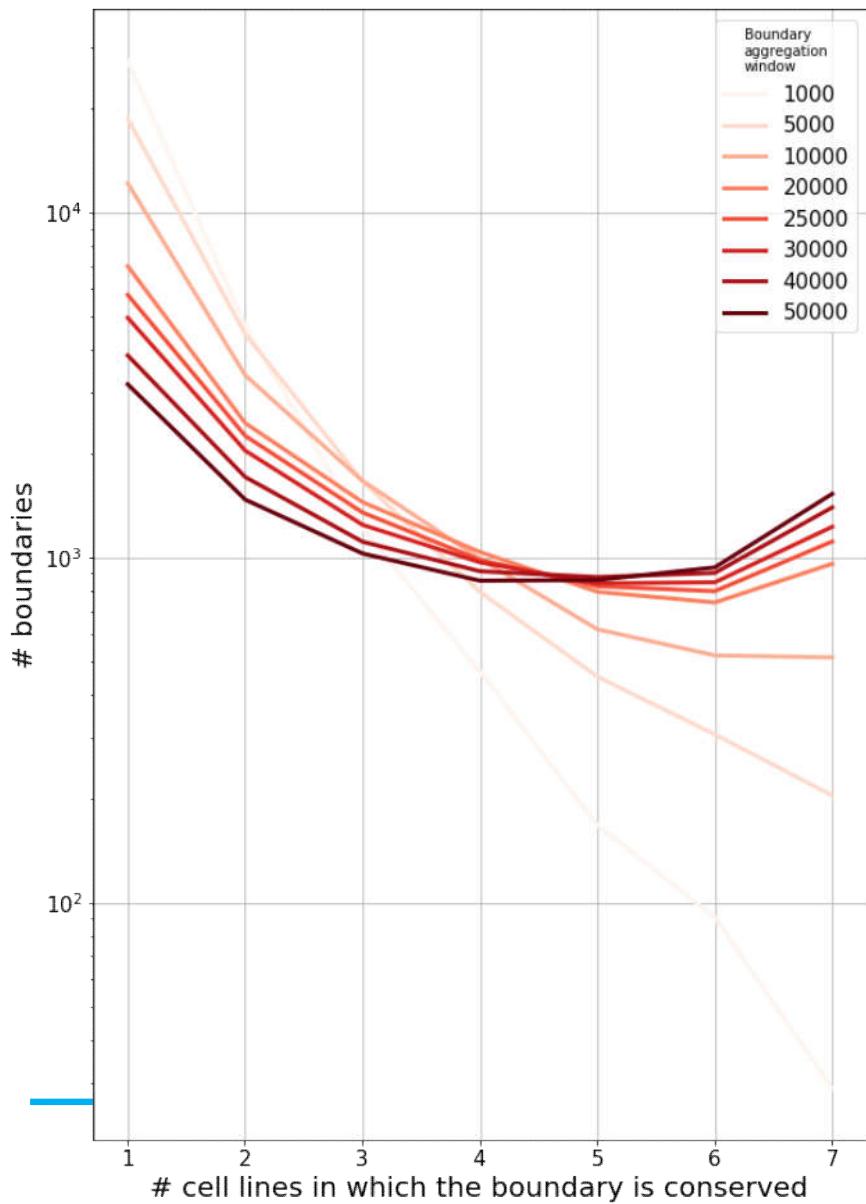
2nm wide

68 nm long

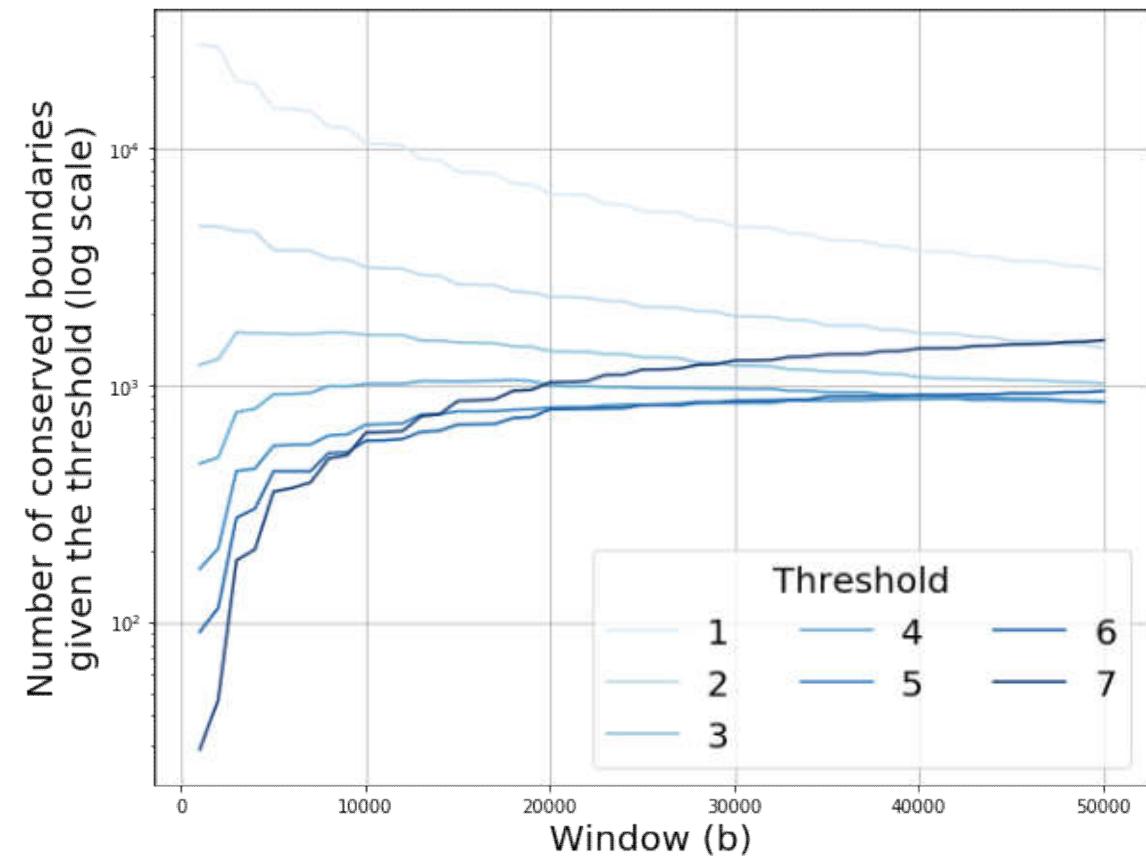
68 μ m long

Nucleus: 5-10 μ m
diameter





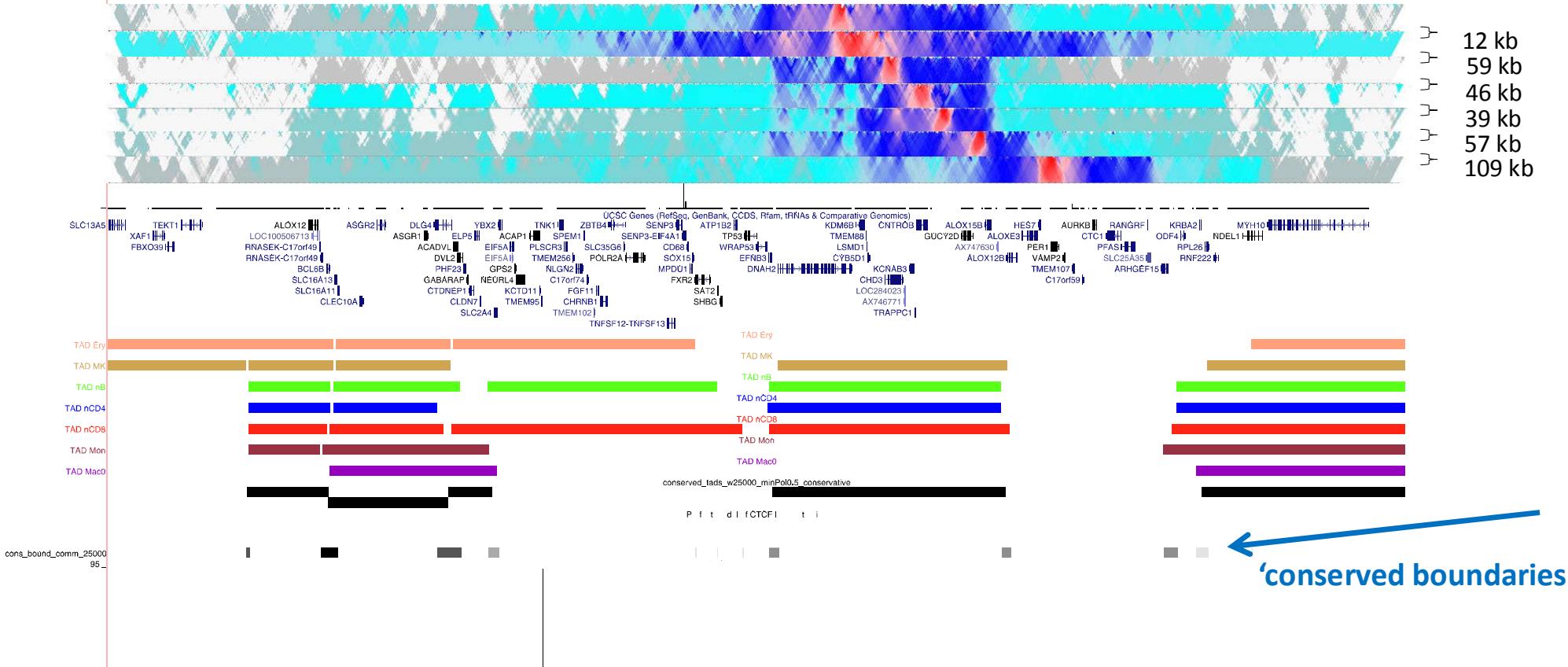
Boundaries seen 4 – 5 times within 25 kb are as ‘good’ as those seen 7 times



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chr17 (p13.1) 17p13.3 17p13.2 17p13.1 17p12 17p11.2 17q11.2 17q12 17q21.2 17q21.31 q21.32 17q21.33 17q22 17q23.2 23.3 q24.1 17q24.2 17q24.3 17q25.1 17q25.3

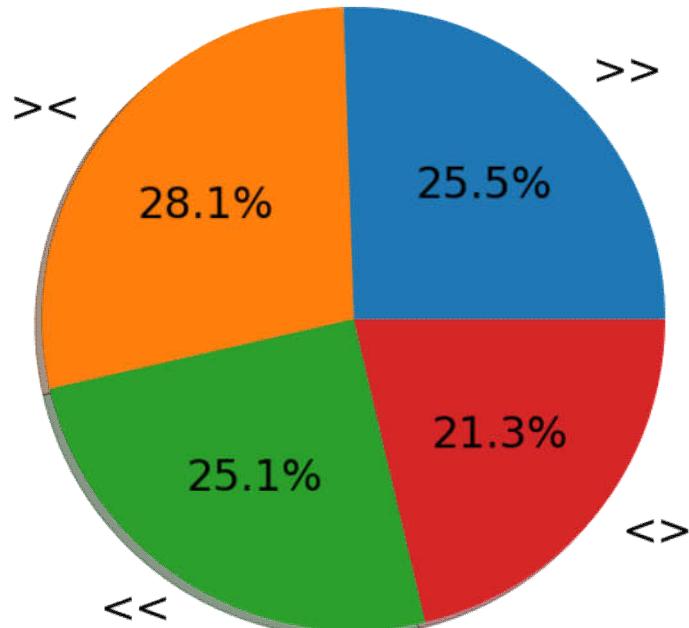


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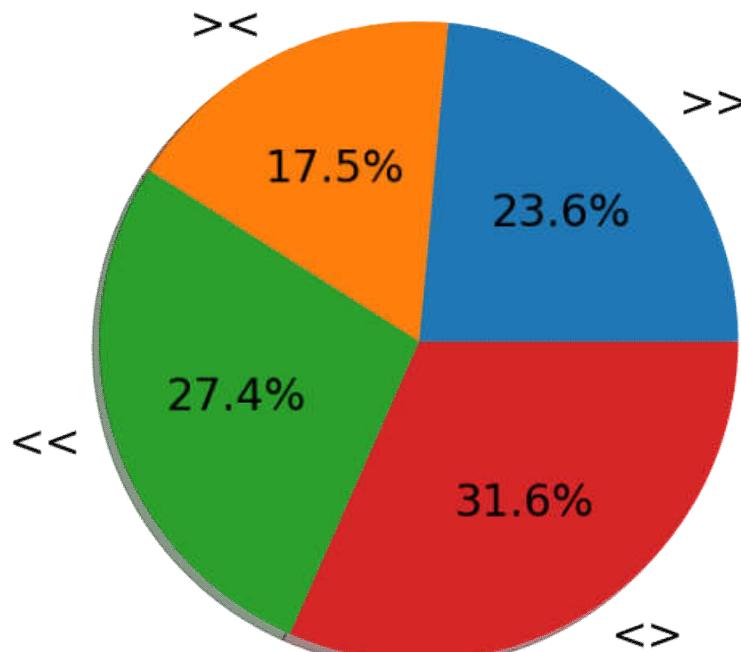
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CTCF-delimited DNA volume is CTCF block class-specific - whole genome versus boundaries

Genome coverage between CTCF patterns



Conserved TAD boundary coverage between CTCF patterns



Di-plets of CTCF

Same (2/4)

>>

<<

Convergent (1/4)

<>

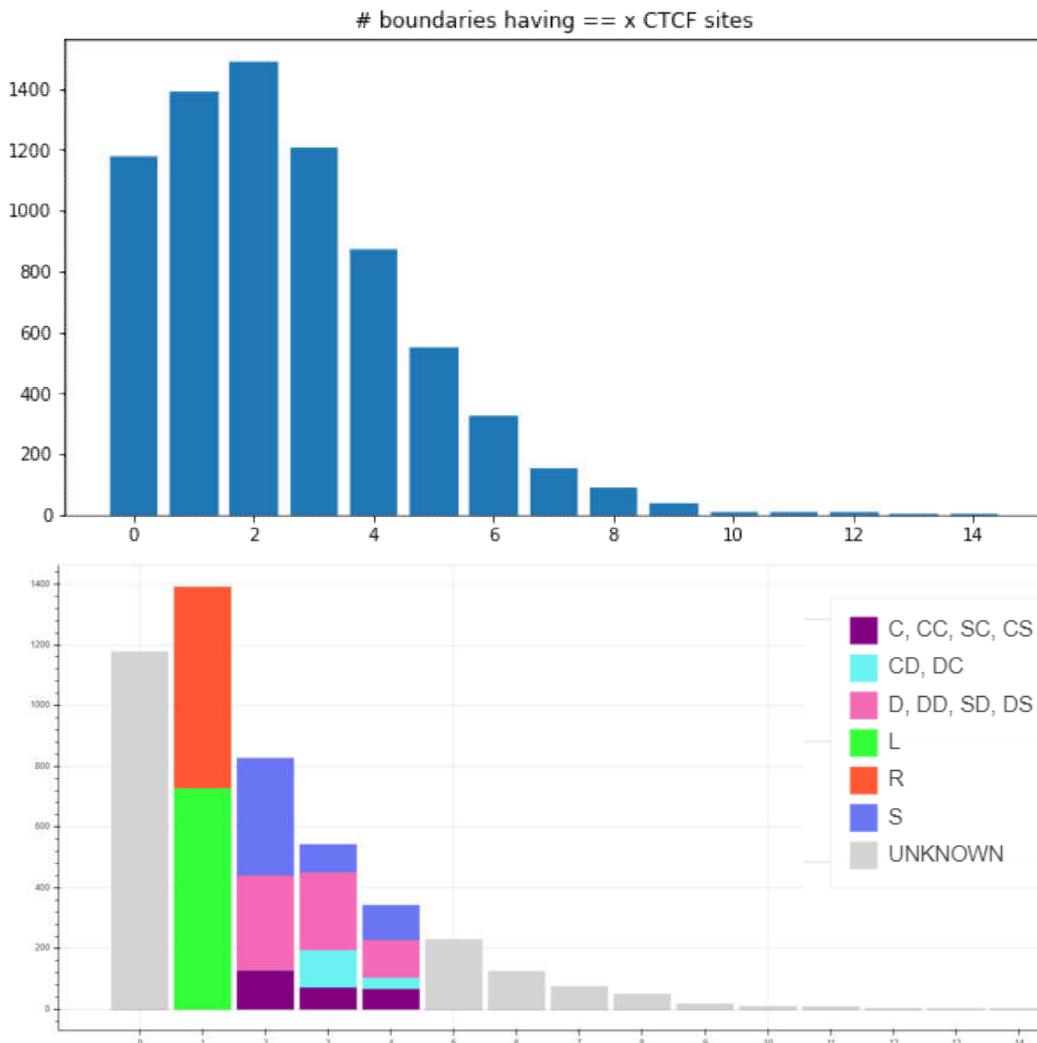
Divergent (1/4)

<>

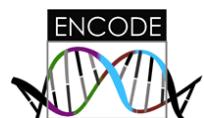


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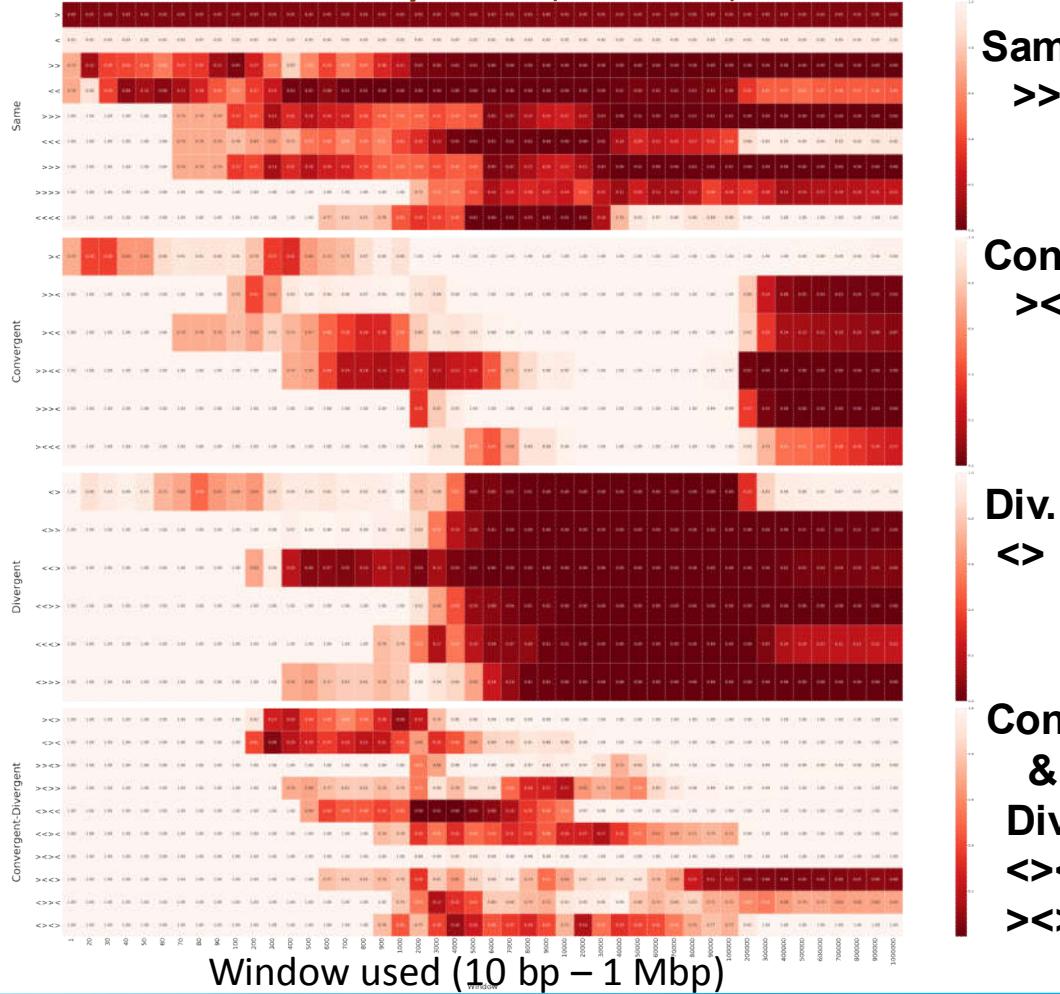
CTCF blocks			
Tetra-	Tri-	Di-	Mono-plets
Same			
>>>	>>	>>	>
<<<	<<<	<<	<
Same-Convergent			
>><	>><	><	
>><	><<		
><<			
Same-Divergent			
<>>	<>>	<>	
<><	<><		
<>>			
Convergent (Same) Divergent			
>><>	><>		
>>>	<><		
<><>			
<><>			
<>><			
<>><			



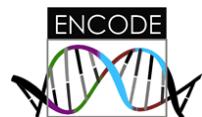
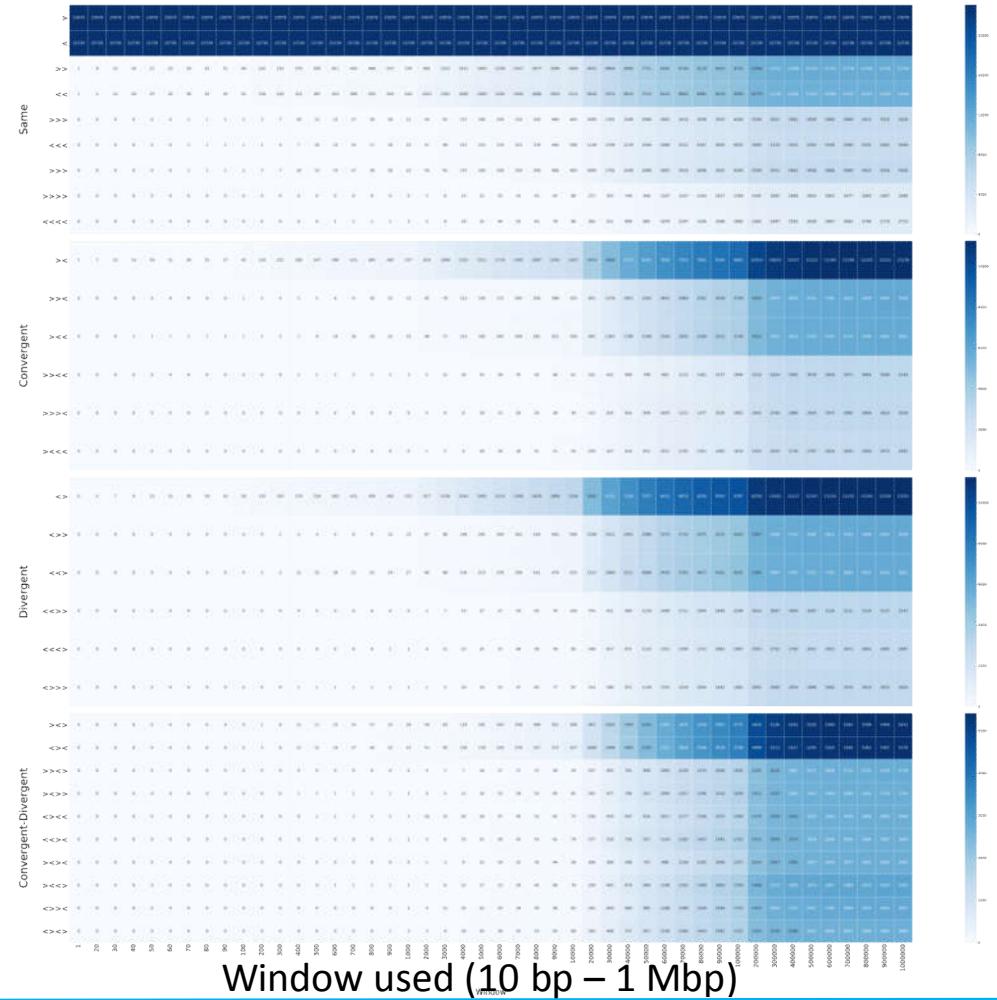
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CTCF blocks, organized by **class** and colored by
binomial test p-value (one-sided)



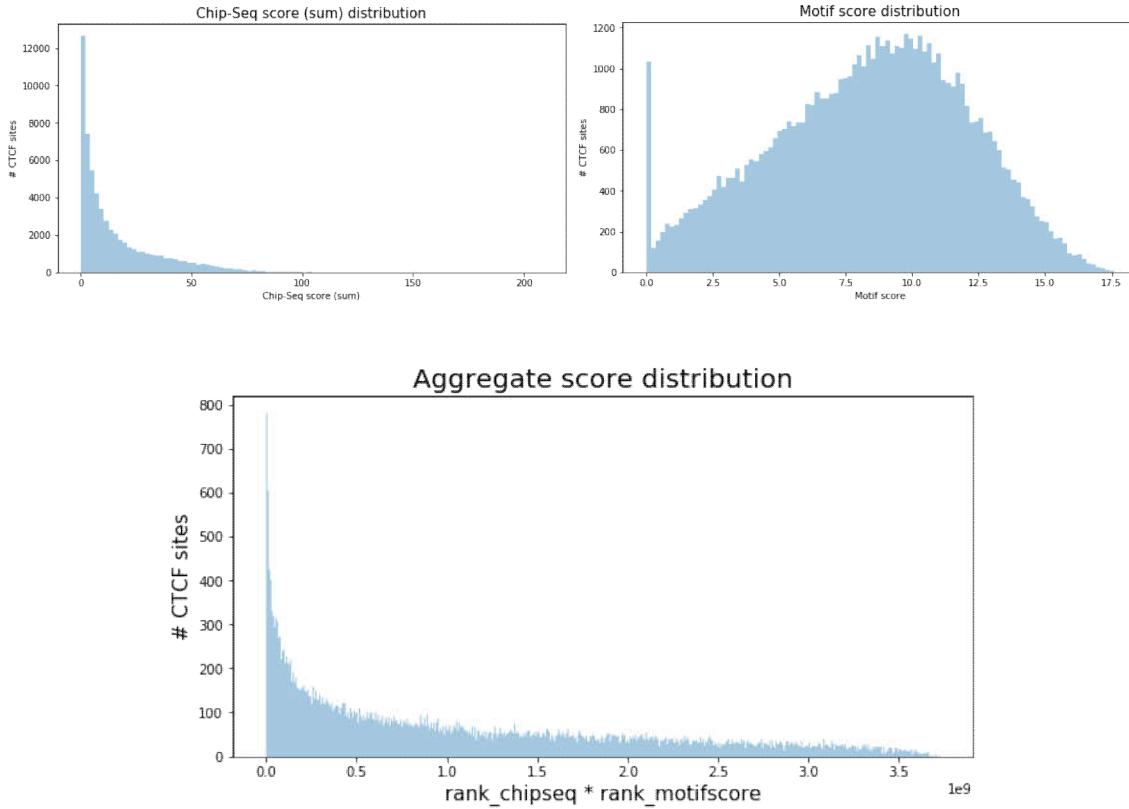
Whole genome counts



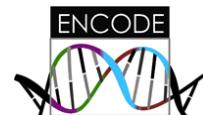
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Including CTCF binding strength: ChIP-seq signal and CTCF motif score

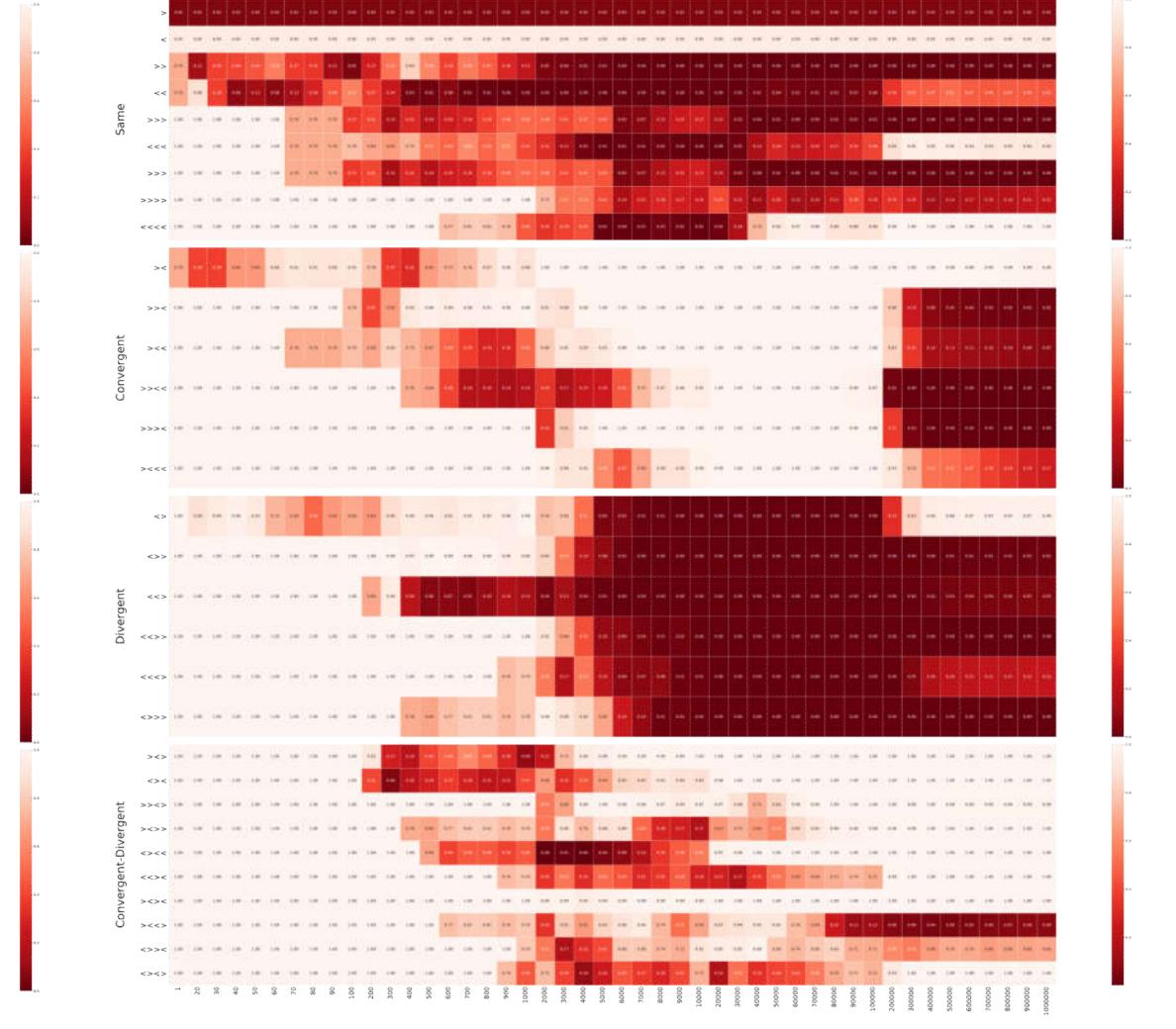
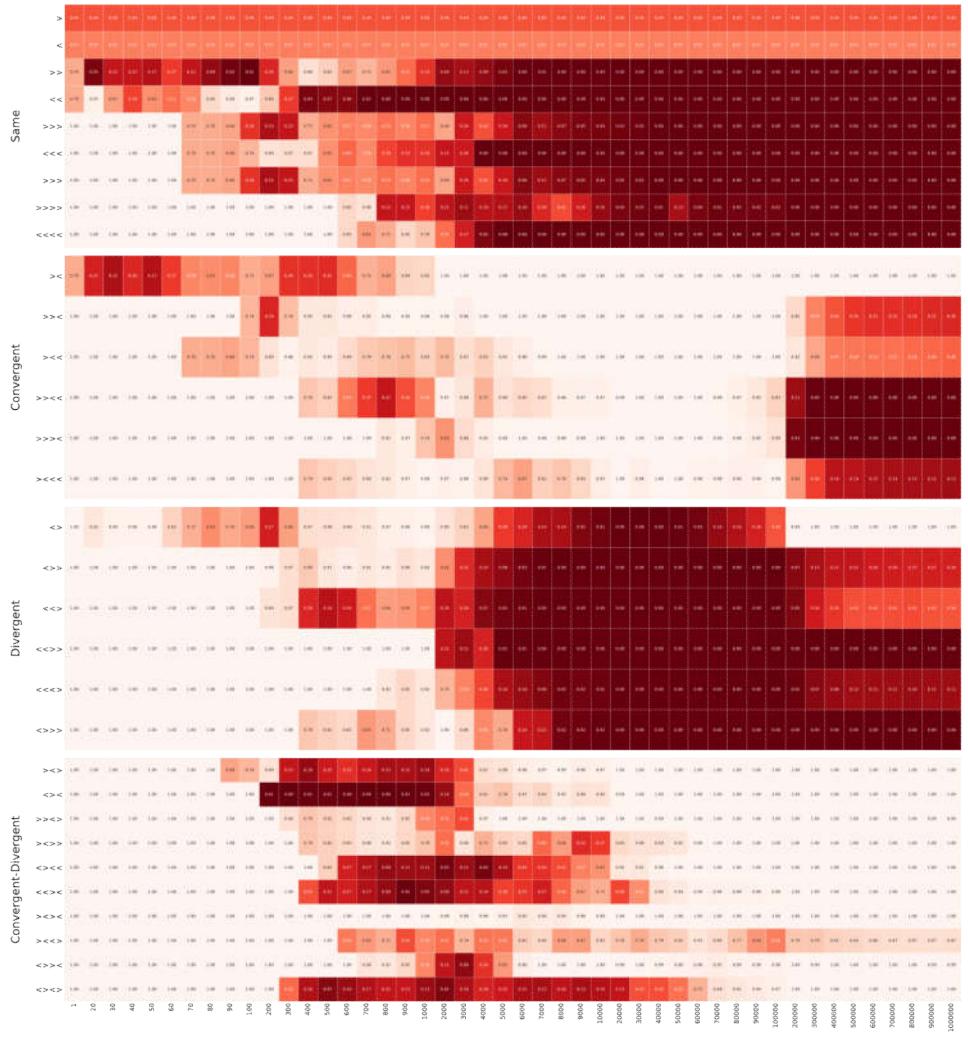


Tetra	Tri-plets	Diplets	Monoplets
Same (2/16)			
>>> Sr-Sr	>>	>>	>
<<< SI-SI	<<<	<<	<
SC (3/16)			
>>< Sr-SI	>><	><	
>>< Sr-C	><<		
><< C-SI			
SD (3/16)			
<>> SI-Sr	<>	<>	
<<< SI-D	<<>		
<>> D-Sr			
CD (8/16)			
>>< Sr-D	><>		
>>< C-Sr	<><		
<>< D-SI			
<>< SI-C			
><< C-C			
><< C-D			
<>< D-C			

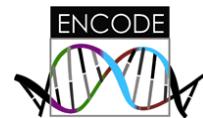
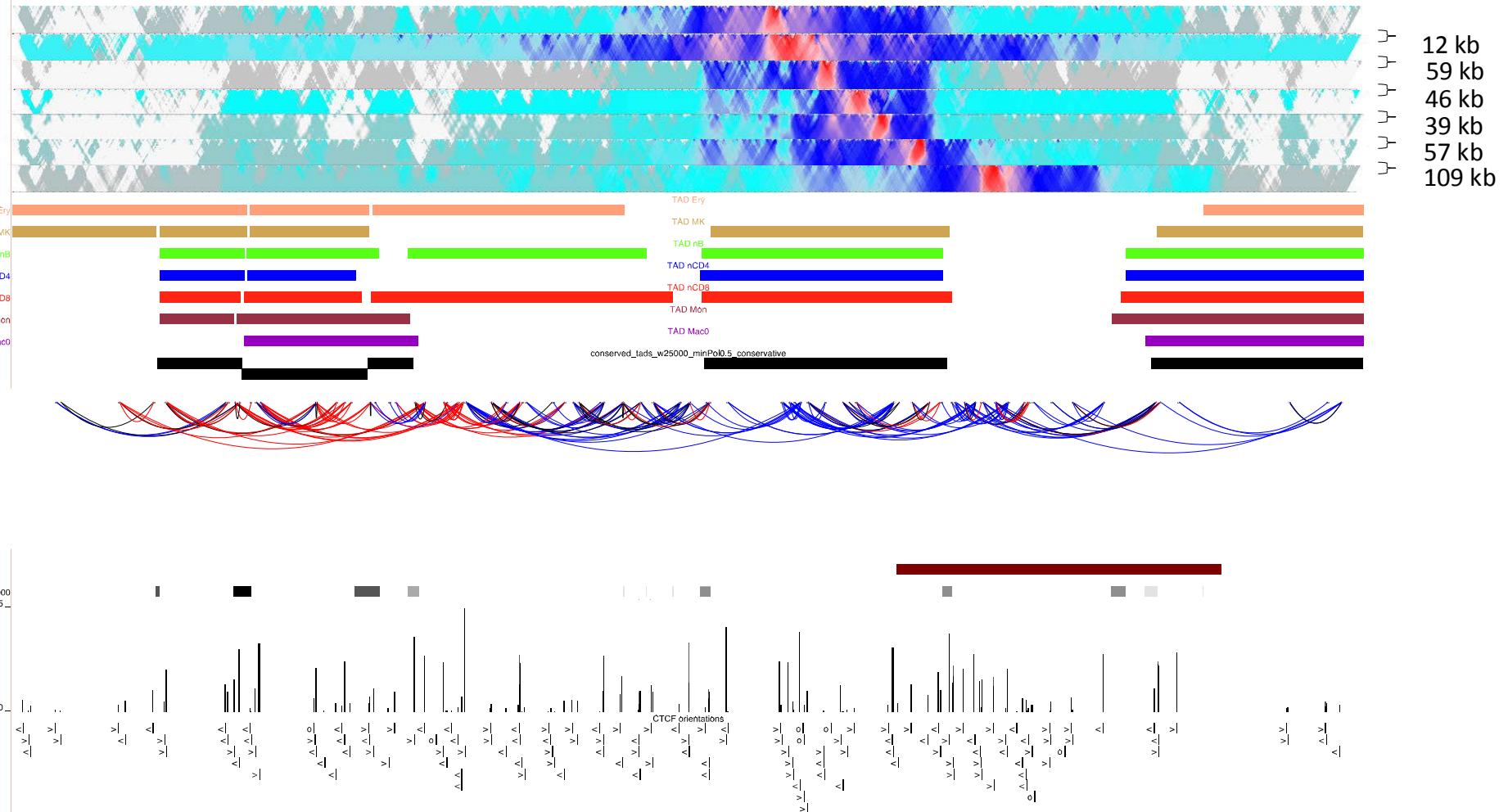


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