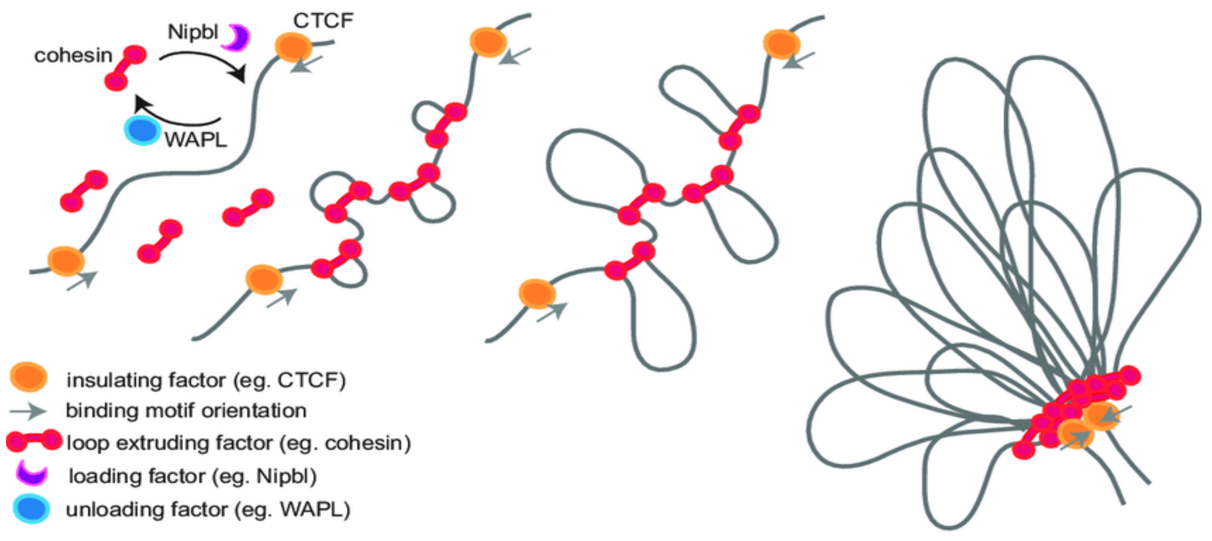
The role of ncRNA in chromatin structure

Sasha Galitsyna
Danila Matveev
Pavel Kuznetsov

CTCF and EZH2 (PRC2) are key proteins of chromatin organization and TAD (Topologically Associating Domains) formation. We hypothesize that ncRNAs can affect this process by acting directly on CTCF and EZH2. We plan to compare data from different Hi-C cell lines with ChIP-seq data on CTCF and EZH2 and then compare ncRNA content with genome-wide ChIP-seq data.

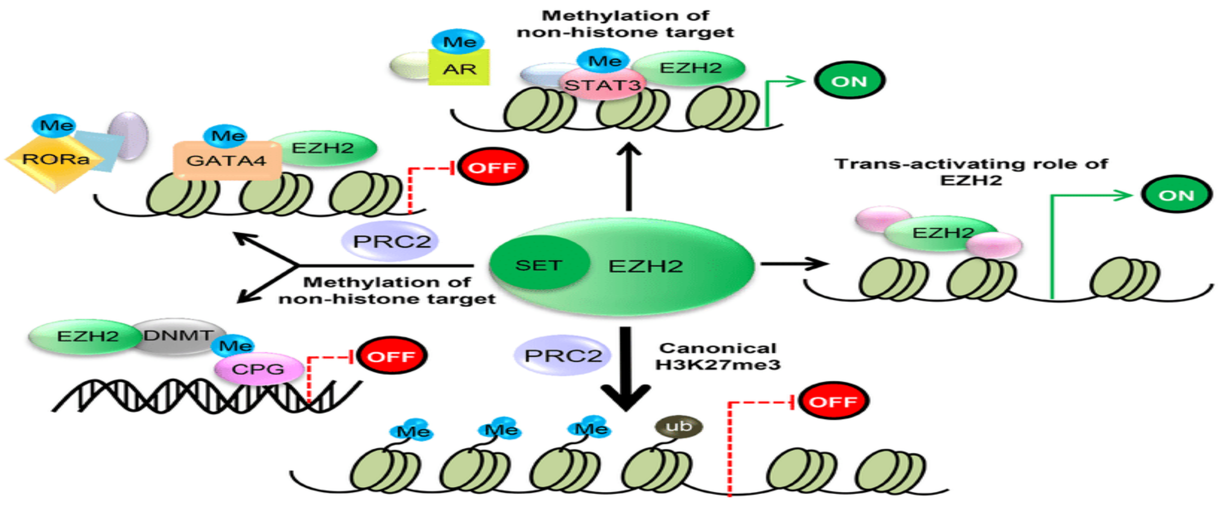
Software list:

- Python
- Numpy
- Bioframe
- Pandas
- Cooler / CoolTools
- HiGlass


cohesin
Nipbl
CTCF
WAPL
insulating factor (eg. CTCF)
binding motif orientation
loop extruding factor (eg. cohesin)
loading factor (eg. Nipbl)
unloading factor (eg. WAPL)

CTCF action model

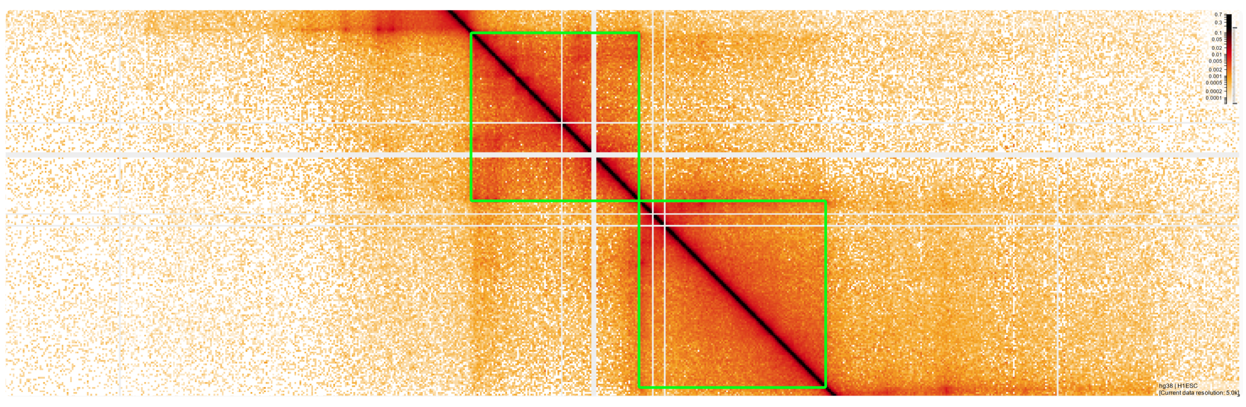
Defining Functionally Relevant Spatial Chromatin Domains: It is a TAD Complicated(Sikorska et al. 2019)- Scientific Figure on ResearchGate



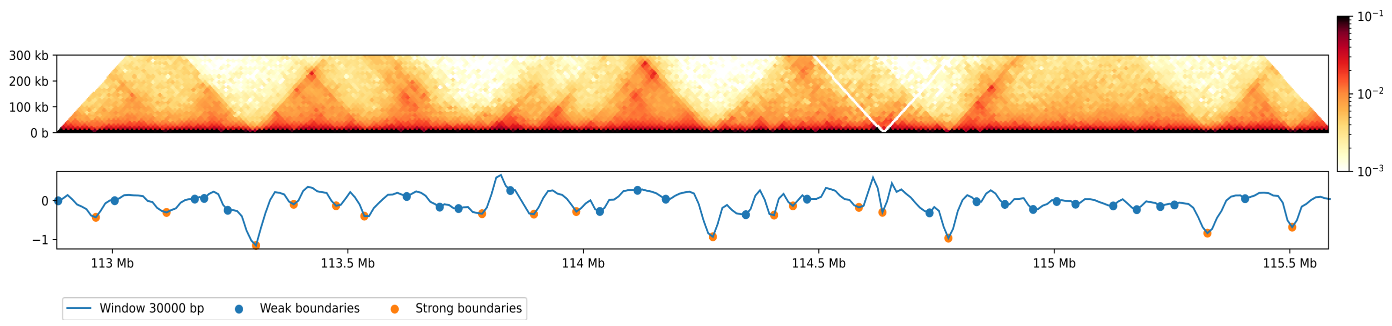
EZH2 action model

Role of EZH2 in cancer stem cells: From biological insight to a therapeutic target (Wen et al. 2015) - Scientific Figure on ResearchGate

Hi-C data from different cell lines (H1ESC, HUVEC, HeLa-S3, IMR-90, K562) were loaded using cooler/cooltools, then domain boundaries were called by finding minimal values on the insulation table. We then used NumPy and Pandas boundaries to divide boundaries into strong and weak, which formed domains with small enrichment of ncRNA because of their size

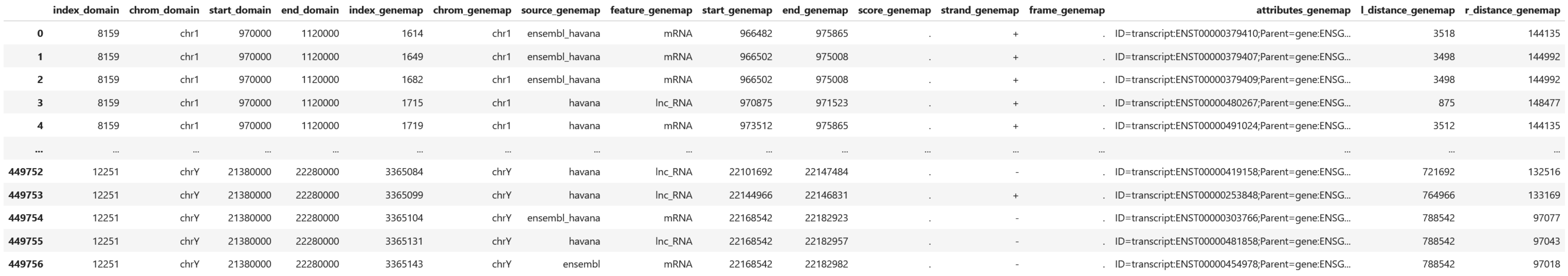


Domains on the Hi-C map (H1ESC; chr11:112,884,917-116,554,270)



Boundaries on the Hi-C map (H1ESC; chr11:112,884,917-116,554,270)

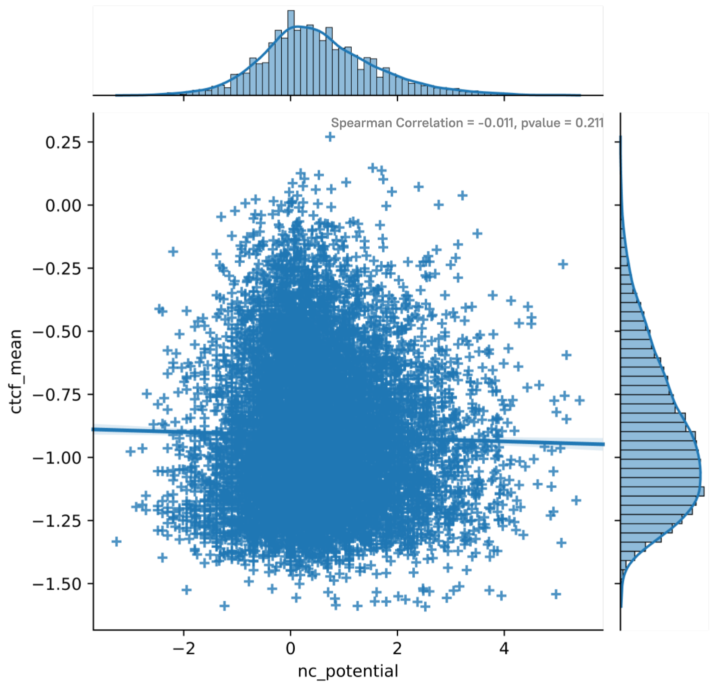
After saving DataFrame with a list of strong domain boundaries, we processed it with bioframe and obtained the final list of domains. We then searched these domains for the presence of different types of RNAs using the Ensembl (ncRNA/mRNA) and LNCipedia (ncRNA) databases. The final result was the table of ncRNA and mRNA for each chromatin domain



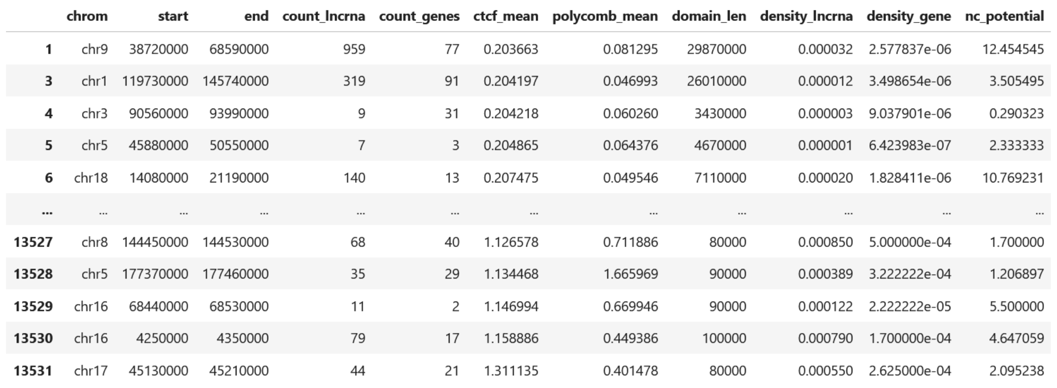
	index_domain	chrom_domain	start_domain	end_domain	index_genemap	chrom_genemap	source_genemap	feature_genemap	start_genemap	end_genemap	score_genemap	strand_genemap	frame_genemap	attributes_genemap	l_distance_genemap	r_distance_genemap
0	8159	chr1	970000	1120000	1614	chr1	ensembl_havana	mRNA	966482	975865	.	+	.	ID=transcript:ENST00000379410;Parent=gene:ENSG...	3518	144135
1	8159	chr1	970000	1120000	1649	chr1	ensembl_havana	mRNA	966502	975008	.	+	.	ID=transcript:ENST00000379407;Parent=gene:ENSG...	3498	144992
2	8159	chr1	970000	1120000	1682	chr1	ensembl_havana	mRNA	966502	975008	.	+	.	ID=transcript:ENST00000379409;Parent=gene:ENSG...	3498	144992
3	8159	chr1	970000	1120000	1715	chr1	havana	lnc_RNA	970875	971523	.	+	.	ID=transcript:ENST00000480267;Parent=gene:ENSG...	875	148477
4	8159	chr1	970000	1120000	1719	chr1	havana	mRNA	973512	975865	.	+	.	ID=transcript:ENST00000491024;Parent=gene:ENSG...	3512	144135
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
449752	12251	chrY	21380000	22280000	3365084	chrY	havana	lnc_RNA	22101692	22147484	.	-	.	ID=transcript:ENST00000419158;Parent=gene:ENSG...	721692	132516
449753	12251	chrY	21380000	22280000	3365099	chrY	havana	lnc_RNA	22144966	22146831	.	+	.	ID=transcript:ENST00000253848;Parent=gene:ENSG...	764966	133169
449754	12251	chrY	21380000	22280000	3365104	chrY	ensembl_havana	mRNA	22168542	22182923	.	-	.	ID=transcript:ENST00000303766;Parent=gene:ENSG...	788542	97077
449755	12251	chrY	21380000	22280000	3365131	chrY	havana	lnc_RNA	22168542	22182957	.	-	.	ID=transcript:ENST00000481858;Parent=gene:ENSG...	788542	97043
449756	12251	chrY	21380000	22280000	3365143	chrY	ensembl	mRNA	22168542	22182982	.	-	.	ID=transcript:ENST00000454978;Parent=gene:ENSG...	788542	97018

DataFrame with mRNA и ncRNA for each domain in the genome (H1ESC)

Using PyBigWig, we processed the ChIP-seq data for CTCF and EZH2, then we calculated the average ChIP-seq values for each domain and downloaded it into a separate table, from which we plotted the ChIP-seq (ctcf_mean/polycomb_mean) with ncRNA density (density_lncrna) in the domain divided by the mRNA density (density_gene) in the domain

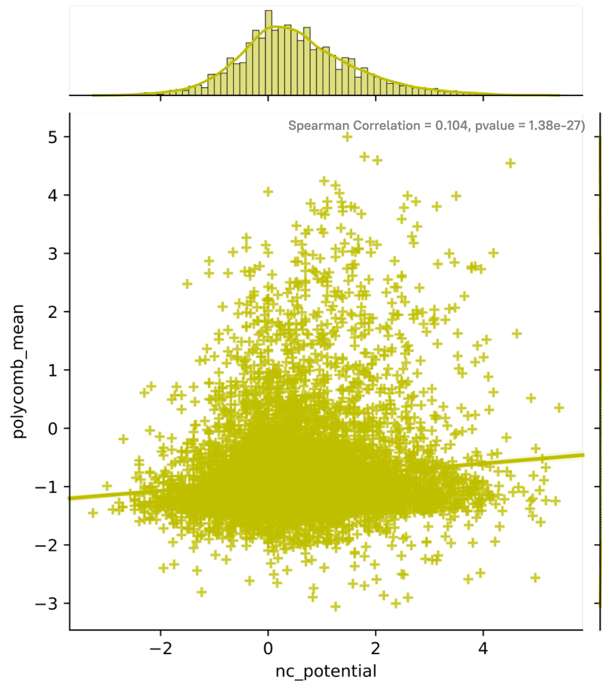


CTCF (H1ESC)



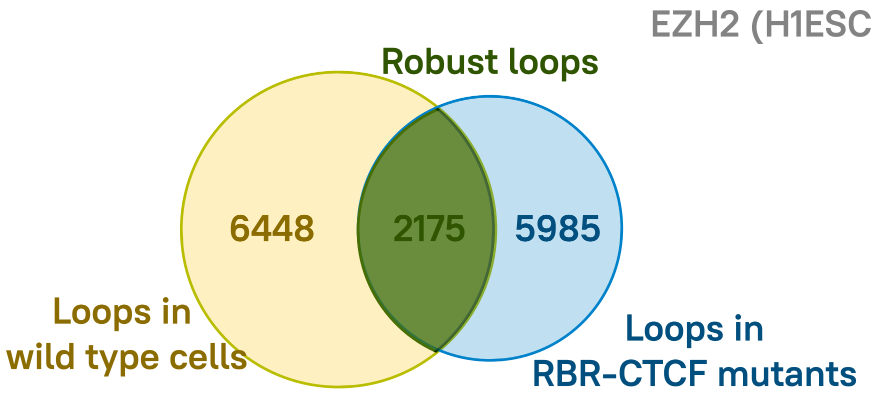
	chrom	start	end	count_lncrna	count_genes	ctcf_mean	polycomb_mean	domain_len	density_lncrna	density_gene	nc_potential
1	chr9	38720000	68590000	959	77	0.203663	0.081295	29870000	0.000032	2.577837e-06	12.454545
3	chr1	119730000	145740000	319	91	0.204197	0.046993	26010000	0.000012	3.498654e-06	3.505495
4	chr3	90560000	93990000	9	31	0.204218	0.060260	3430000	0.000003	9.037901e-06	0.290323
5	chr5	45880000	50550000	7	3	0.204865	0.064376	4670000	0.000001	6.423983e-07	2.333333
6	chr18	14080000	21190000	140	13	0.207475	0.049546	7110000	0.000020	1.828411e-06	10.769231
...	...	...	...	...	...	...	...	...	...	...	...
13527	chr8	144450000	144530000	68	40	1.126578	0.711886	80000	0.000850	5.000000e-04	1.700000
13528	chr5	177370000	177460000	35	29	1.134468	1.665969	90000	0.000389	3.222222e-04	1.206897
13529	chr16	68440000	68530000	11	2	1.146994	0.669946	90000	0.000122	2.222222e-05	5.500000
13530	chr16	4250000	4350000	79	17	1.158886	0.449386	100000	0.000790	1.700000e-04	4.647059
13531	chr17	45130000	45210000	44	21	1.311135	0.401478	80000	0.000550	2.625000e-04	2.095238

Plotting DataFrame (H1ESC)

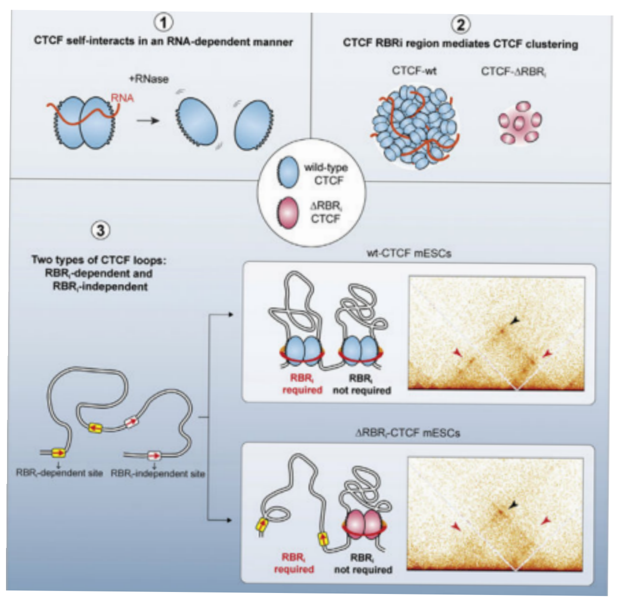


EZH2 (H1ESC)

CTCF is a DNA-binding protein having RNA-binding region (RBR). Recently, it became known that chromatin structure depends on the localization of CTCF in DNA, and on the binding of RNA. Taking Hi-C data for wild-type and RBR-CTCF mutant mouse cells, we decided to explore this dependence in more detail.

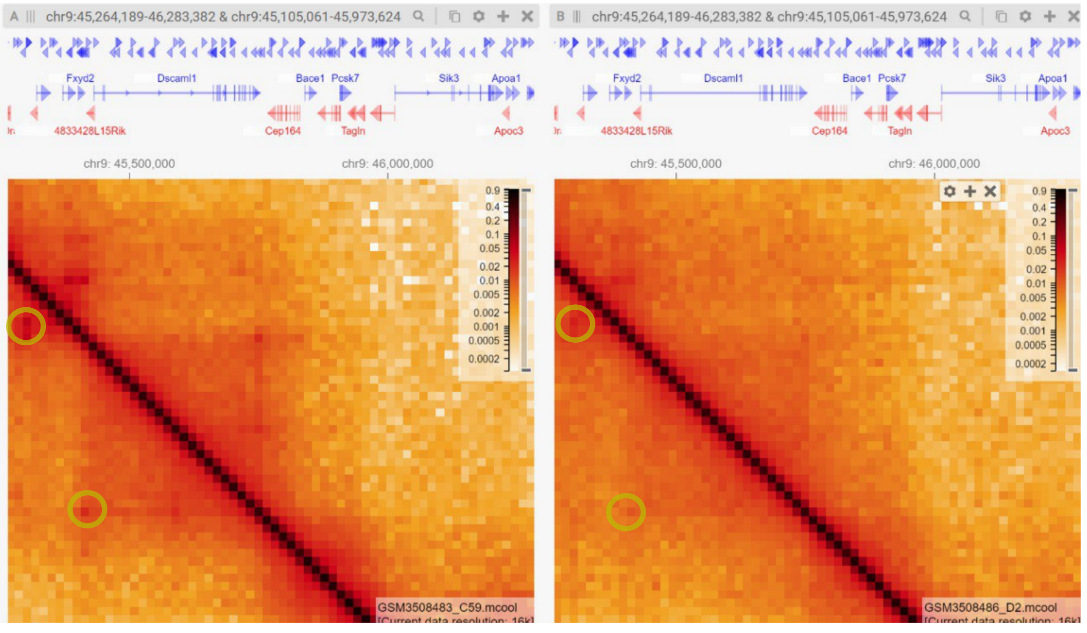


Types of CTCF-dependent loops

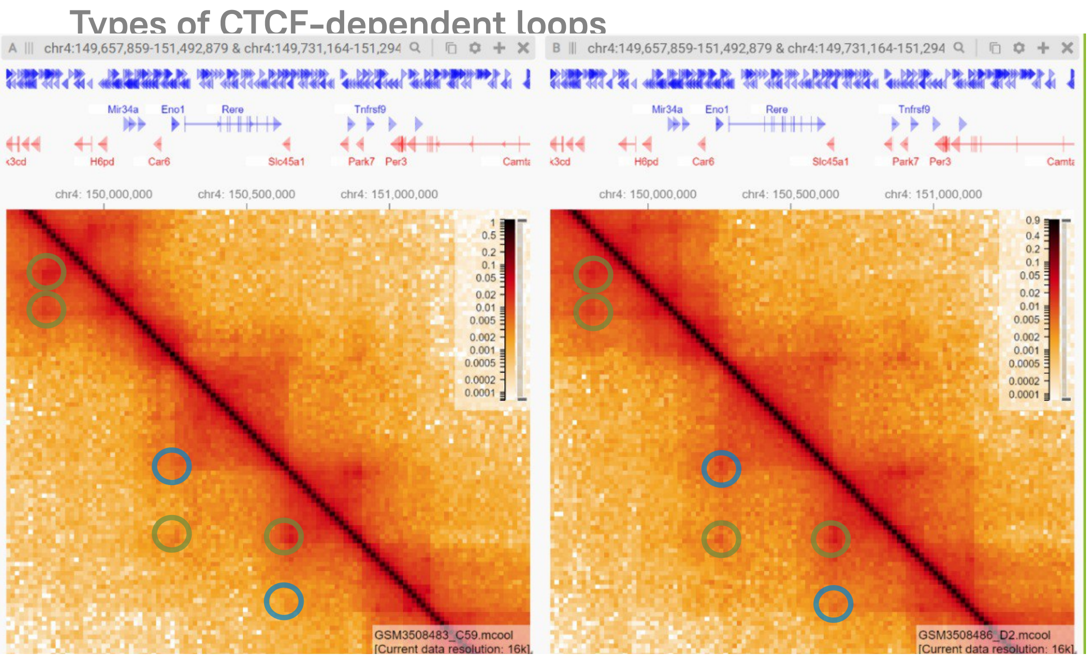


CTCF mechanism model

Distinct Classes of Chromatin Loops Revealed by Deletion of an RNA-Binding Region in CTCF (Hansen et al. 2019)



Loops at chromosome 9



Loops of different types at chromosome 4

hhmi Howard Hughes Medical Institute
SMTB
ZIMIN FOUNDATION