Players and Models of Transcription Regulation in 3D genome

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The Jackson Laboratory for Genomic Medicine
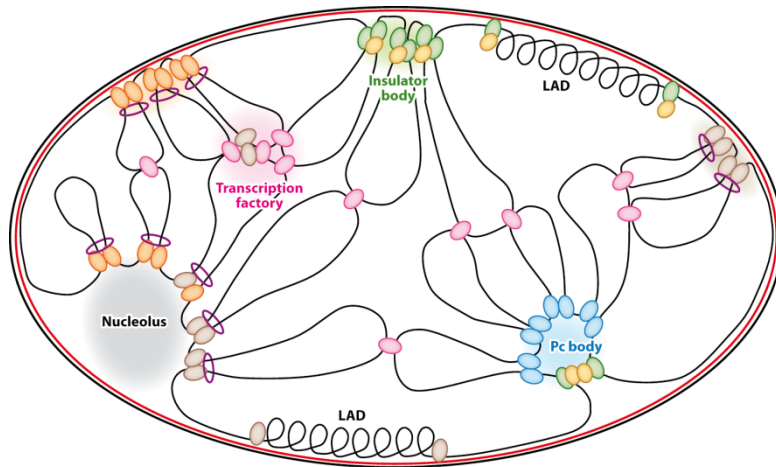**Department of Genetics and Genome Sciences, UConn Health Center**

Players and Models of Transcription Regulation in 3D genome

The players: DNA, Protein, RNA

Protein-mediated chromatin interactions

RNA-mediated chromatin interactions

- TF
- Cohesin/condensin
- CTCF
- TFIIIC
- Pc
- CP190/Mod(mdg4)
- SuHw/dCTCF/BEAF
- Nuclear lamina

Van Bortle K, Corces VG. 2012.
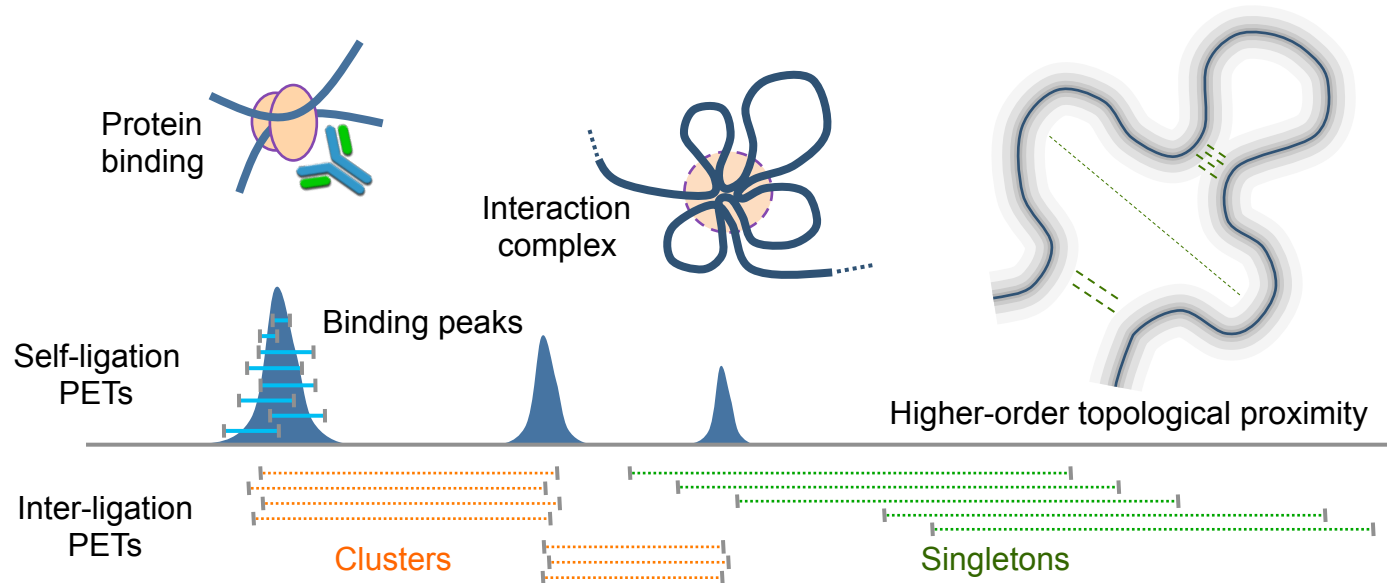Annu. Rev. Cell Dev. Biol. 28:163–87
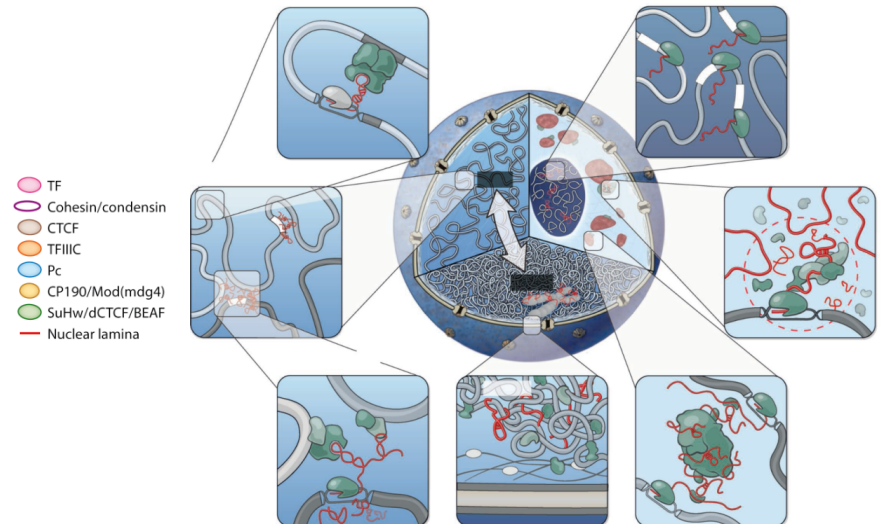
Rinn and Guttman Science 2014

3D Nucleome

ChIA-PET for 3D genome mapping

(multiplex datasets)

Inclusive:
- **Protein bindings,**
- **Enriched chromatin interaction,**
- **Non-enriched contacts (Hi-C like data)**

High specificity and resolution:
- **Functional element specific,**
- **Haplotype resolved,**
- **Single nucleotide resolution**

Fullwood et al 2009 *Nature*; Li et al 2012 *Cell*; Tang et al et al 2015 *Cell*
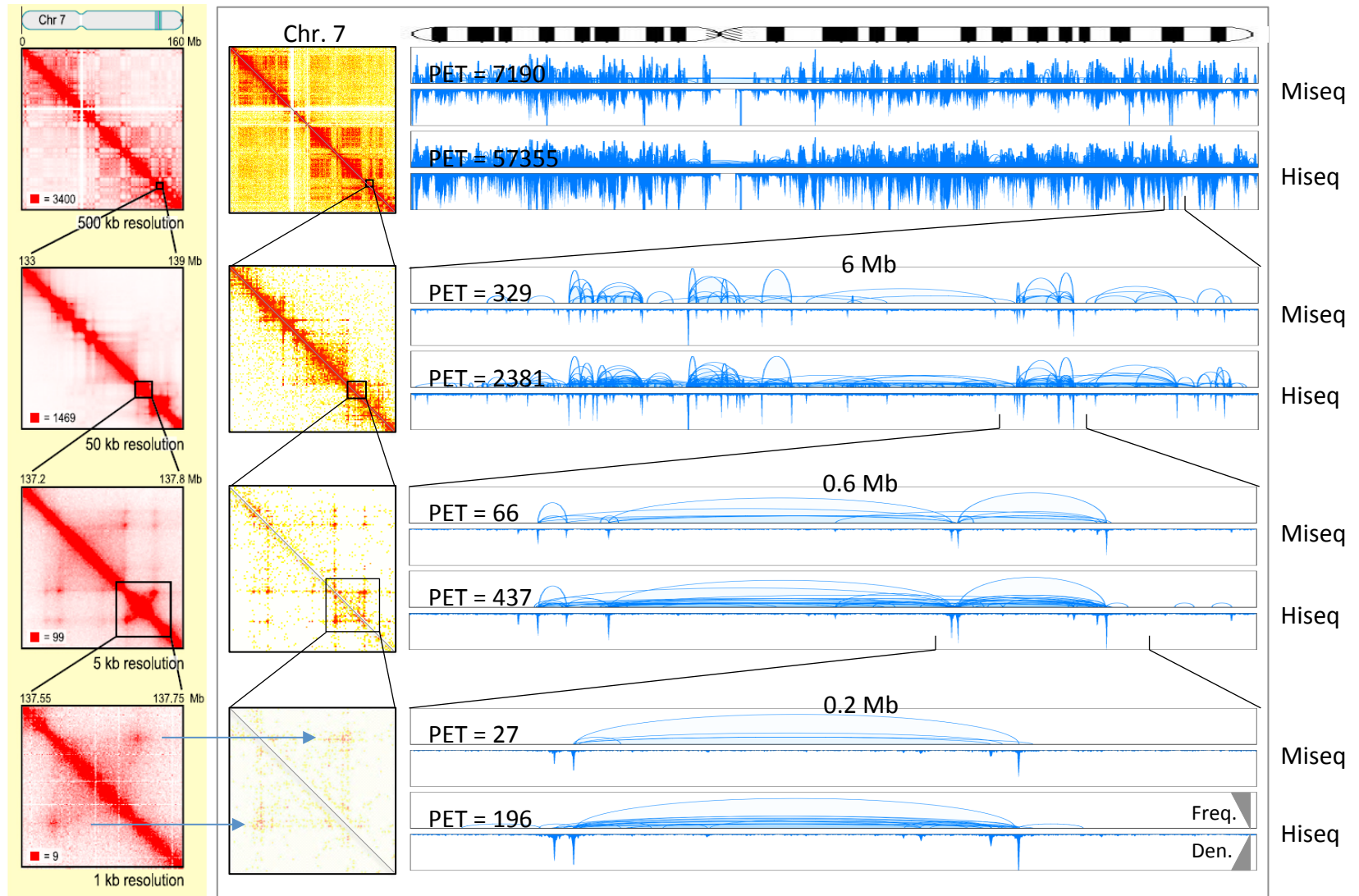
In situ Hi-C   vs.   CTCF ChIA-PET   (GM12878)

Total contact reads = 4.9 Billion

Total PET reads: 1 Miseq = 6.4 Million; 1 Hiseq = 30.8 Million

Rao et al 2014 *Cell*   Tang et al 2015 *Cell*

Connecting loops to form <u>C</u>TCF <u>C</u>ontact <u>D</u>omains (CCD)

Double knot

chr4:109556994-113054287 (3.5Mb)

Freq.

1000

CTCF loops

CCD

CTCF motifs

Den.

600

CTCF peaks

Hi-C TAD

in situ Hi-C loops

ChromHMM

100

100

RNA-Seq
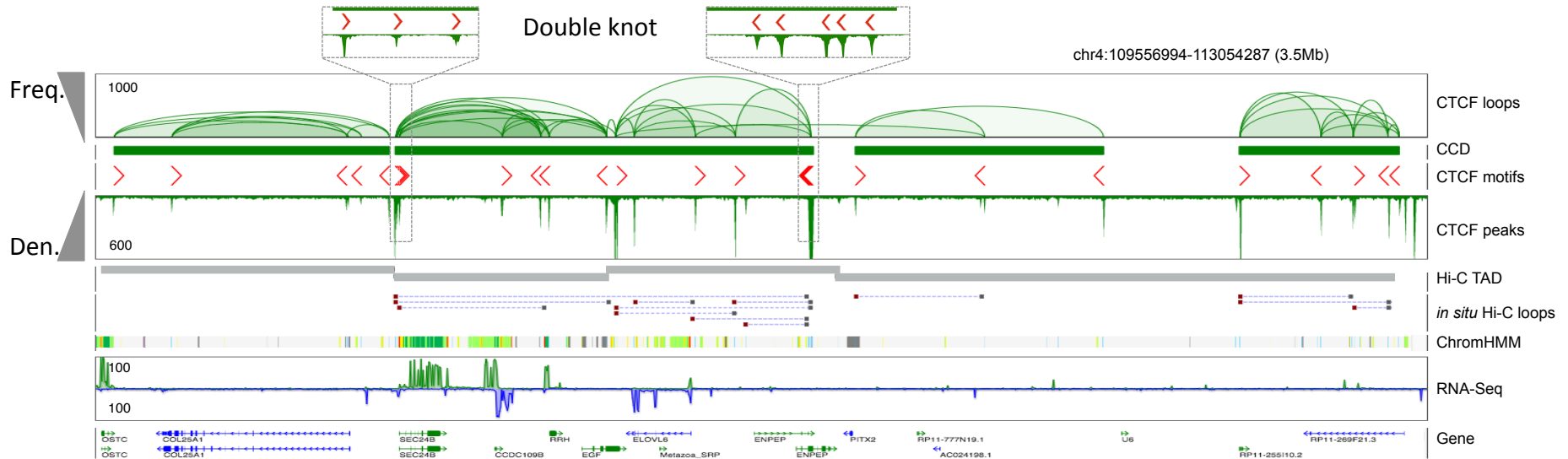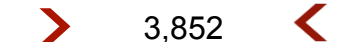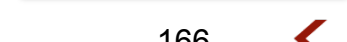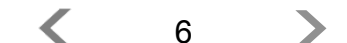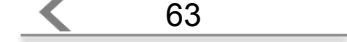
Gene

Size distribution of loop and domain

CCD   2,267

Boundaries
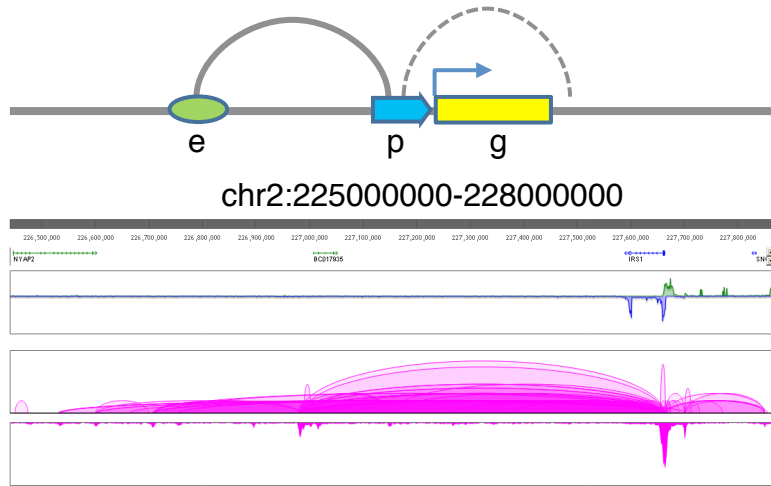
Inward motifs
- 3,852
- 159
- 166
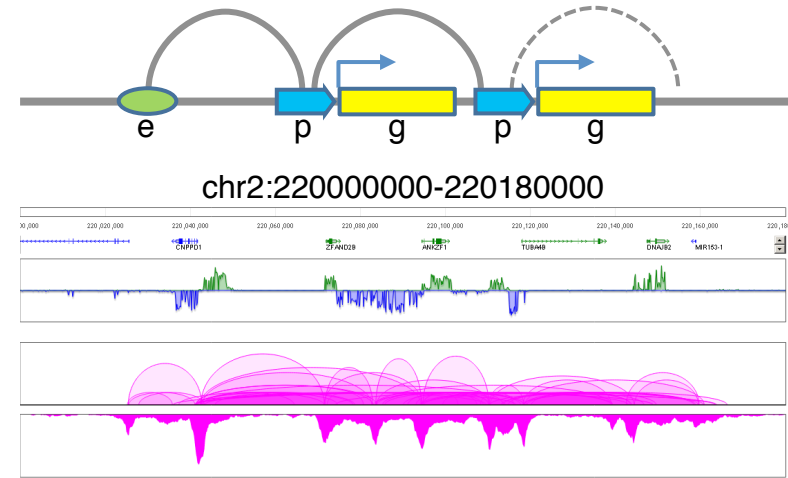
4,177 (97%)

Outward motifs
- 6
- 63
- 56

125 (3%)
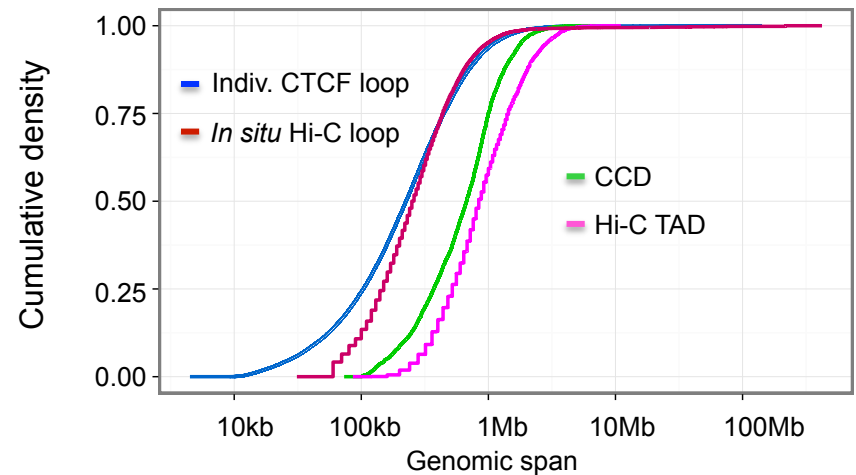
CCD = TAD

RNAPII associated chromatin interactions
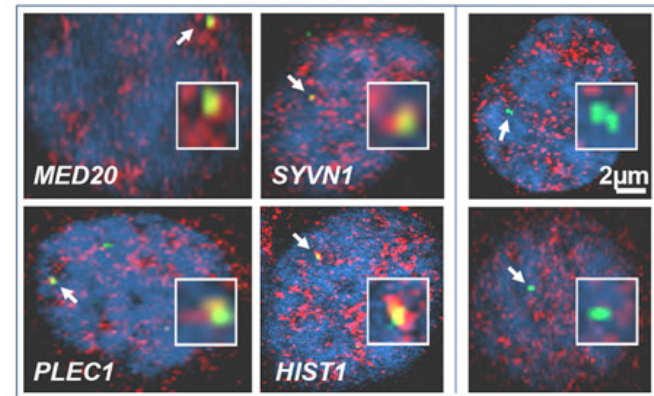
Extra-genic enhancer-promoter interaction (EP)
Single gene complex

Inter-genic promoter-promoter interaction (PP)
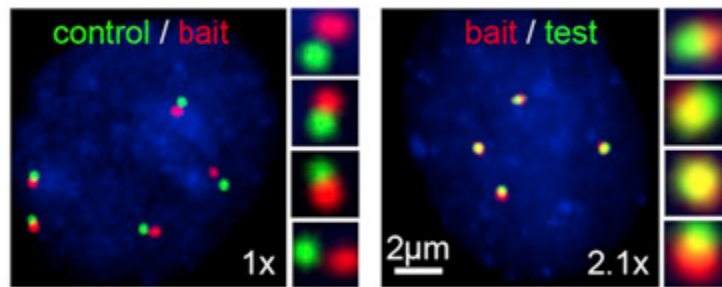Multi-gene complex

chr2:225000000-228000000

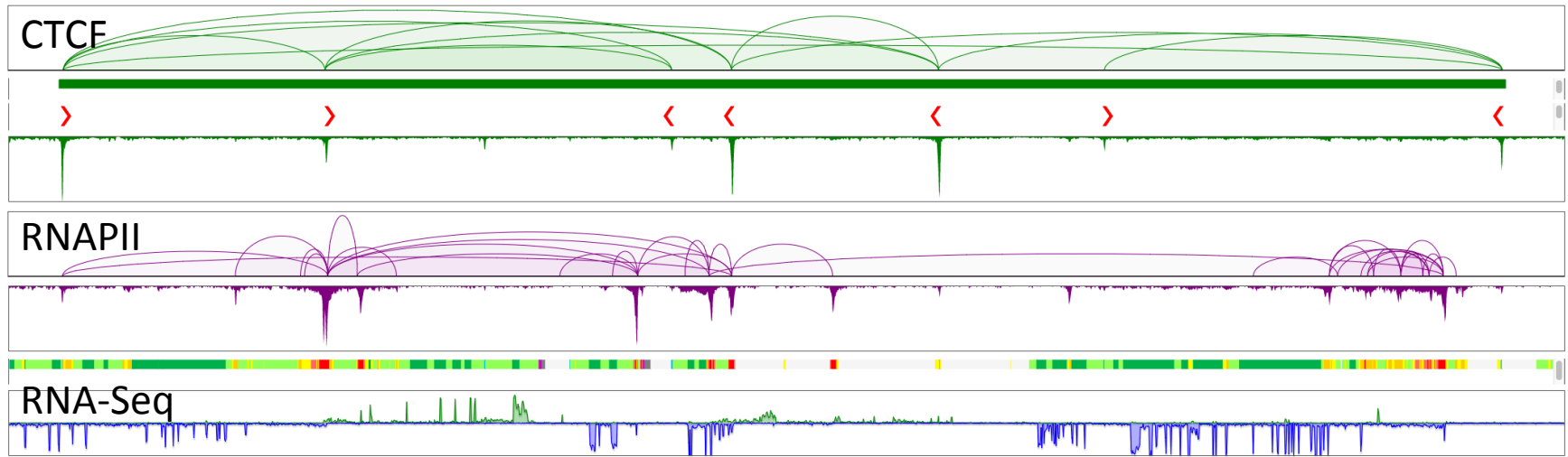chr2:220000000-220180000

RNAPII / DNA

control / bait   bait / test

n =341   n = 368

MED20   SYVN1

PLEC1   HIST1

MG1-4 experiments   Control

Li et al 2012 Cell

CCD

2D mapping data

CTCF

RNAPII

RNA-Seq

3D simulation

3D visualization

E

5 µm

0.5 µm

CTCF RNAPII DNA   Colocalization

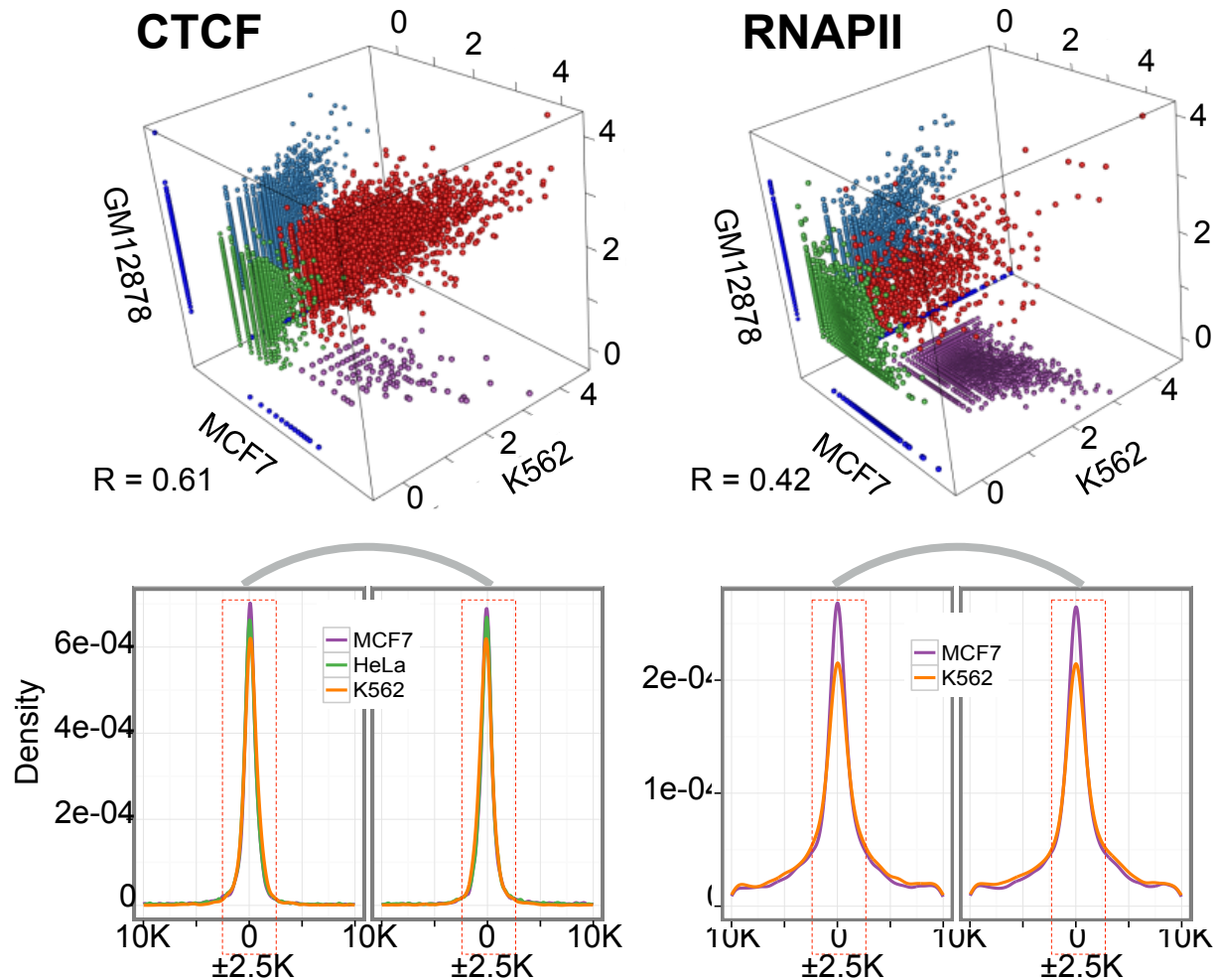Przemyslaw Szala, Dariusz Plewczynski
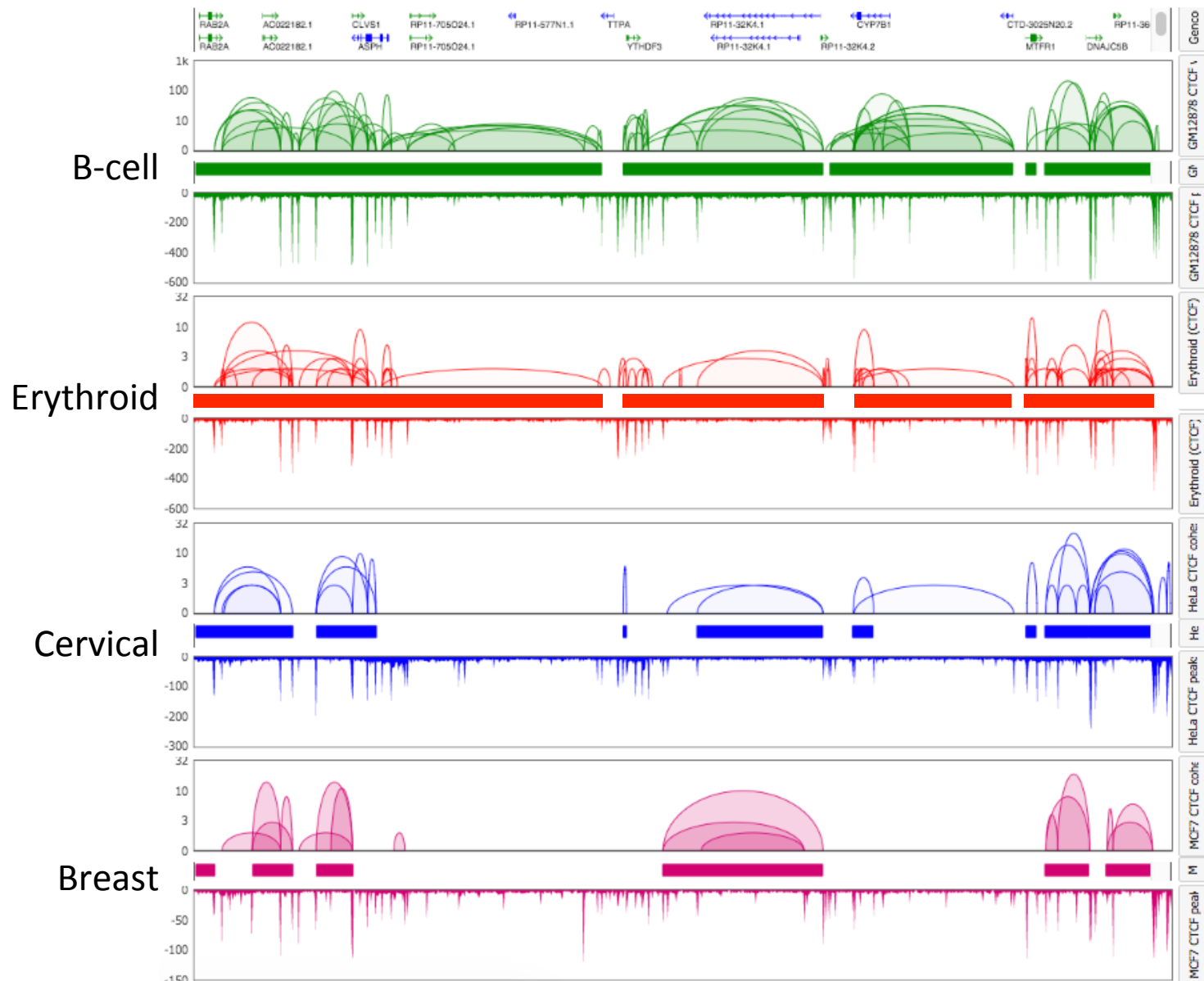
Pawel Trzaskoma, Grzegorz M. Wilczynski,
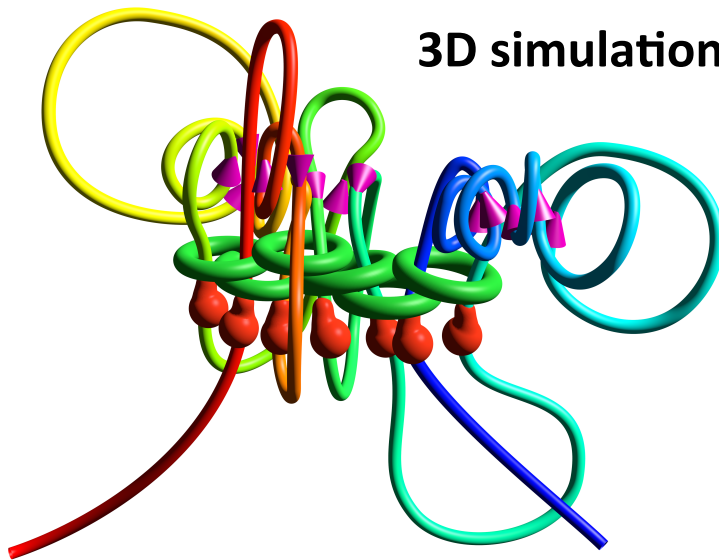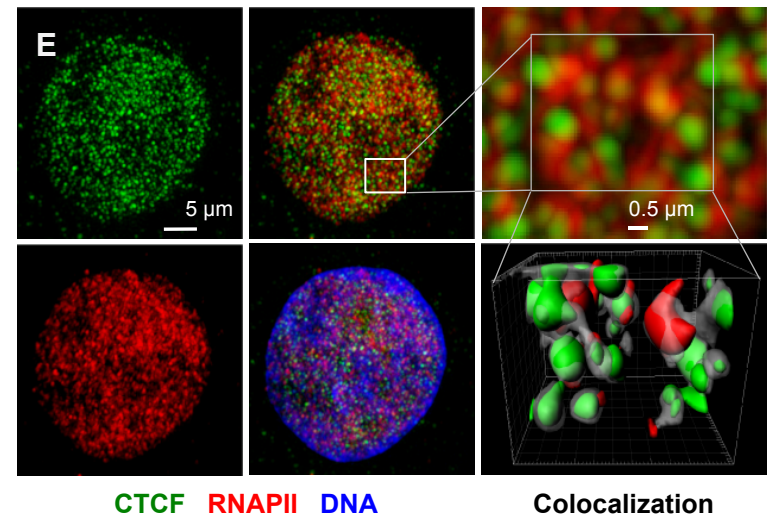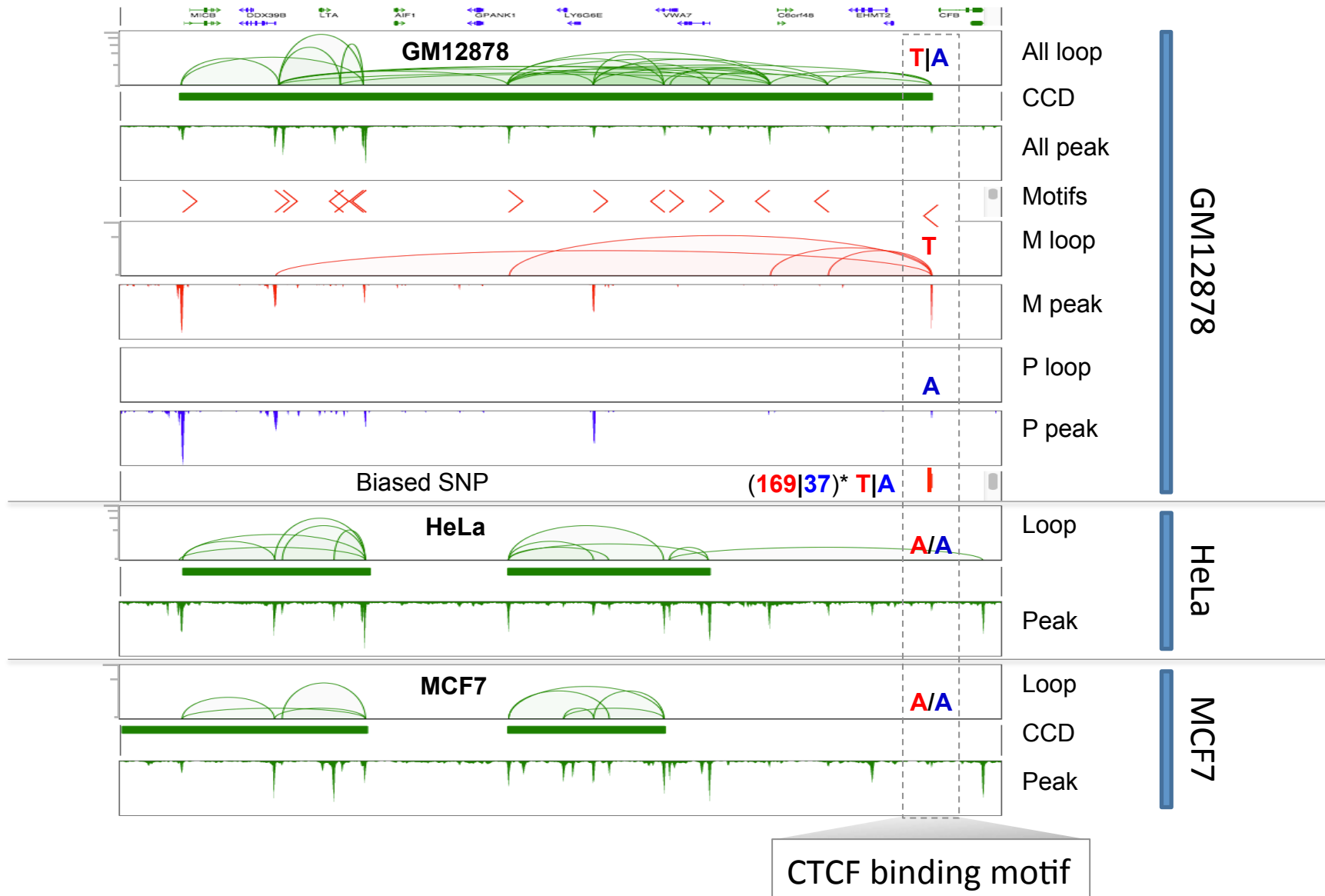
Common & cell-type specific chromatin looping structures

Common & cell type-specific CCD structure
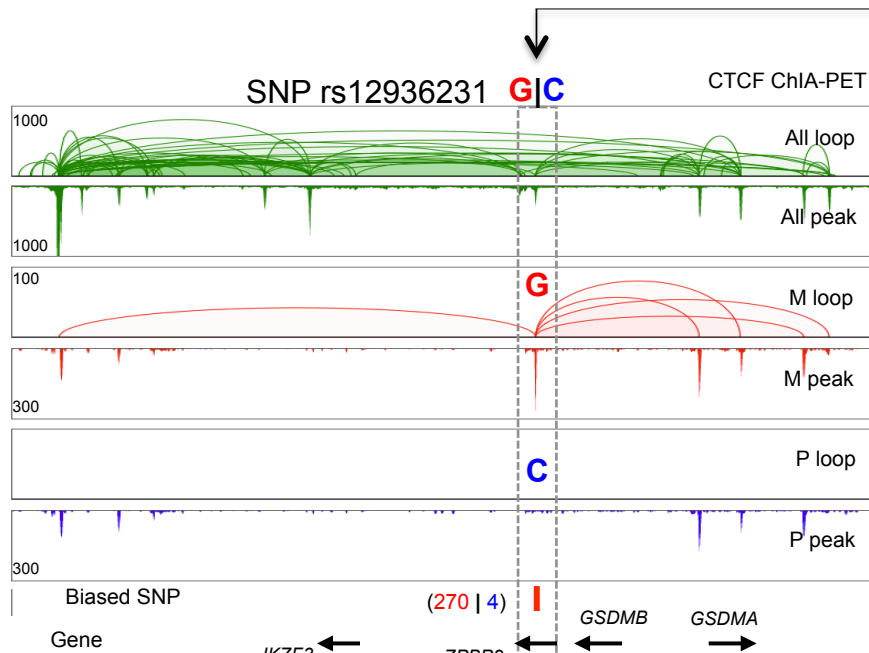
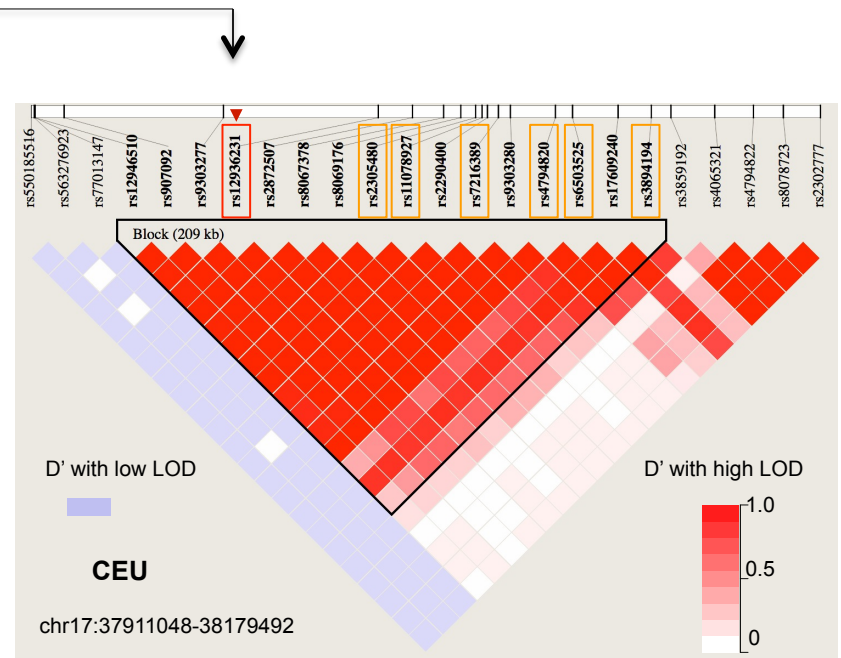# Genetic variations alter chromatin domains

chr6:31426075-31930740 (504 kb)

CTCF binding motif
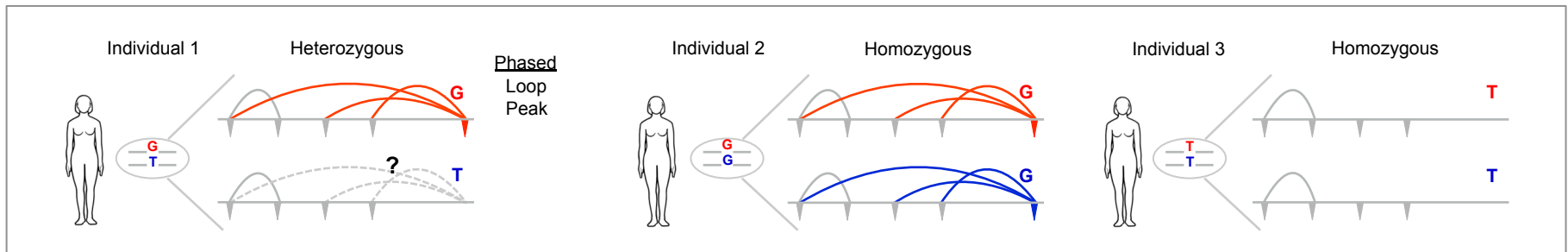
Haplotype-resolved interactions linked to genetics

SNP rs12936231 G|C — CTCF ChIA-PET

All loop
All peak
M loop
M peak
P loop
P peak
Biased SNP (270 | 4)
Gene   IKZF3   ZPBP2   GSDMB   GSDMA   ORMDL3

M: AGTTACTTACATTAGCCCCCAGATGGAGTGAAACCATCAAGTA
P: AGTTACTTACATTAGCCCCCAGATGCAGTGAAACCATCAAGTA

Block (209 kb)
D' with low LOD   D' with high LOD
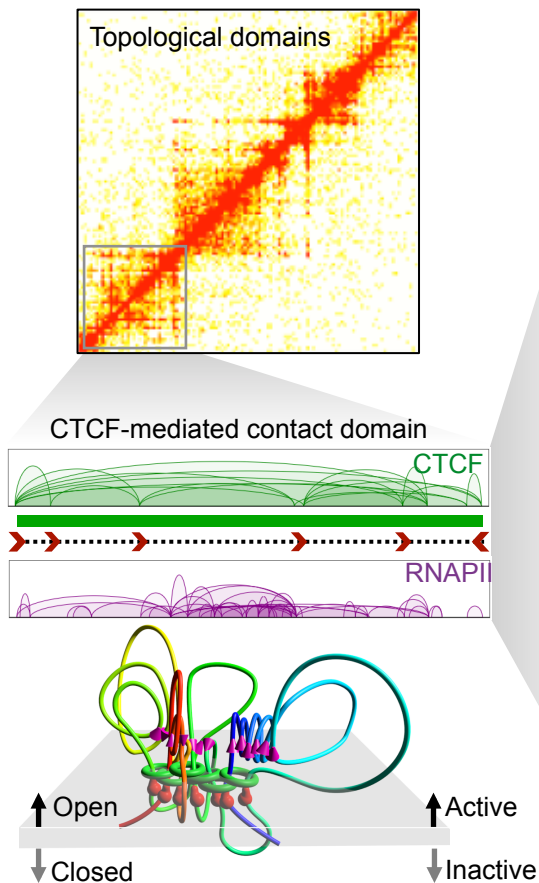CEU
chr17:37911048-38179492

High-risk SNPs for asthma and autoimmune disease alter domain-wide transcription of certain genes (Verlaan 2009)
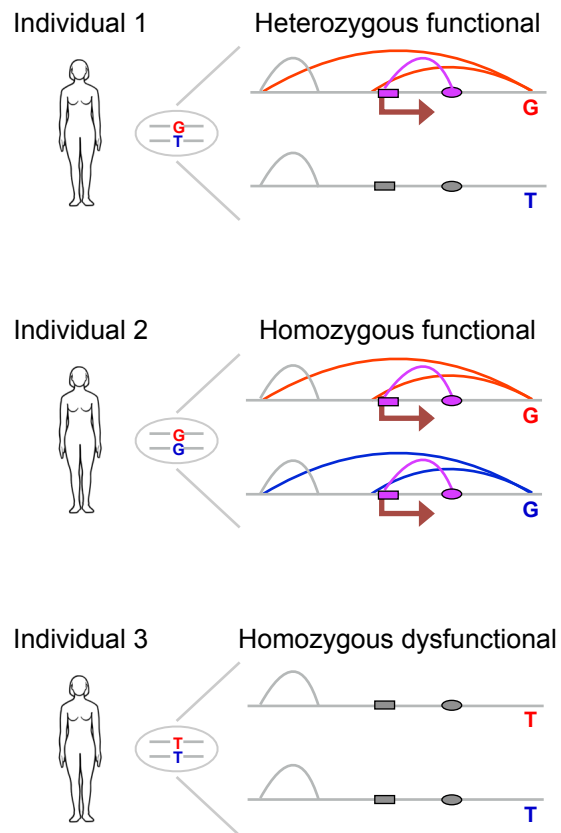
SNP-based validation of CTCF binding and looping

Individual 1 — Heterozygous — Phased Loop Peak
Individual 2 — Homozygous
Individual 3 — Homozygous

3D Genome Structure → Genome Function

3D chromatin architecture

Topological domains

CTCF-mediated contact domain

CTCF

RNAPII

↑Open
↓Closed

↑Active
↓Inactive

Haplotype chromatin interaction

Individual 1 — Heterozygous functional
G
T
G
T

Individual 2 — Homozygous functional
G
G
G
G

Individual 3 — Homozygous dysfunctional
T
T
T
T
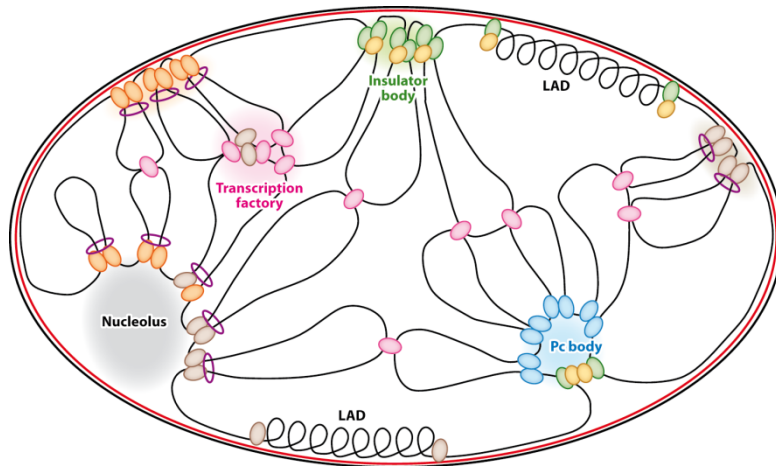
Genetic variations → [ ] **→ Traits/diseases**

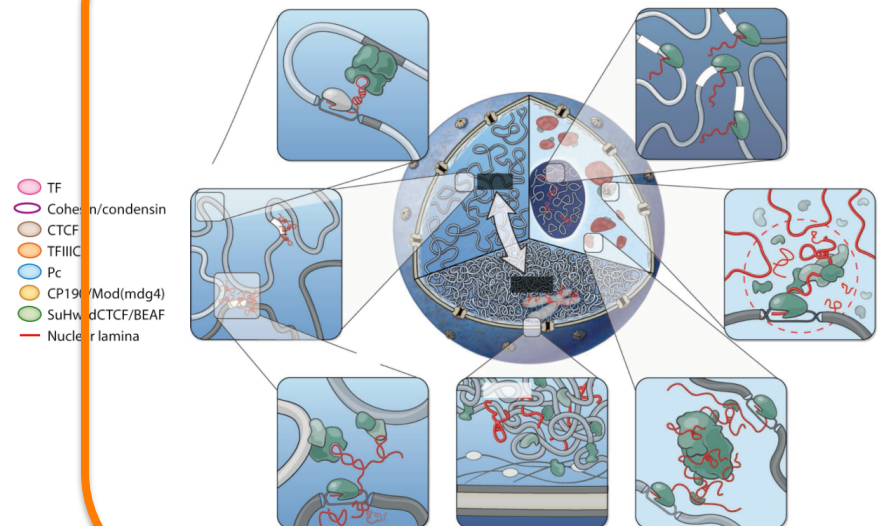# Players and Models of Transcription Regulation in 3D genome

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Protein-mediated chromatin interactions

Van Bortle K, Corces VG. 2012.
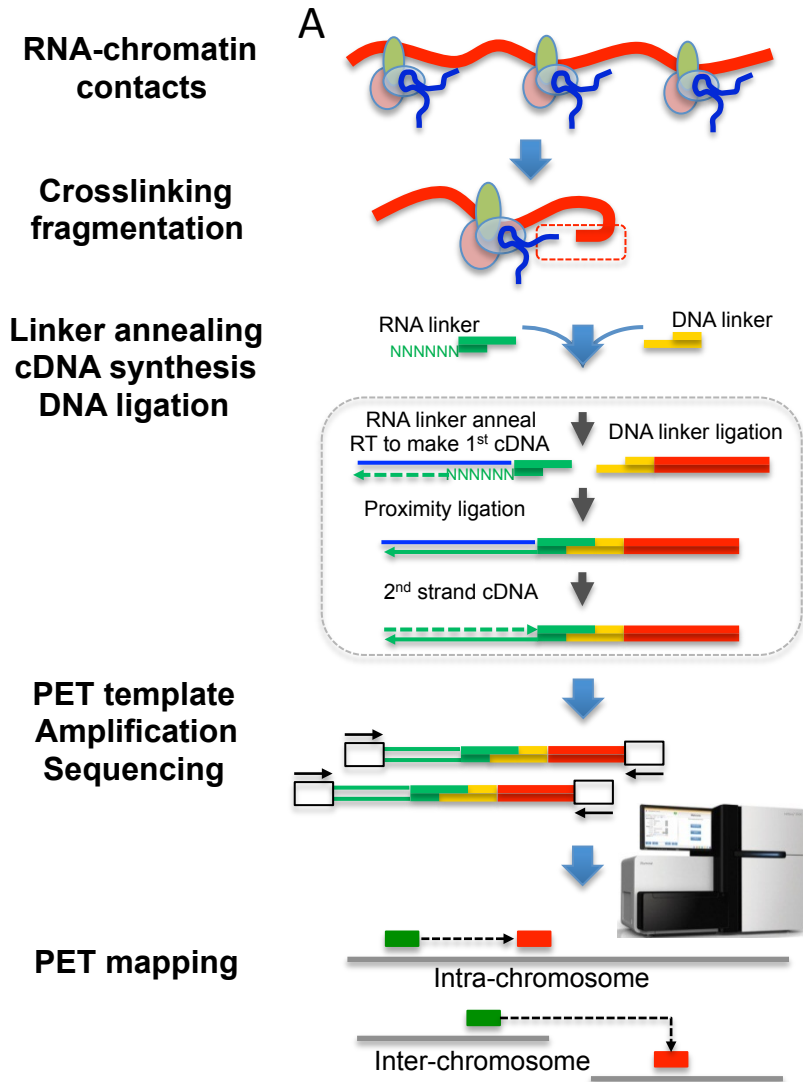Annu. Rev. Cell Dev. Biol. 28:163–87

RNA-mediated chromatin interactions

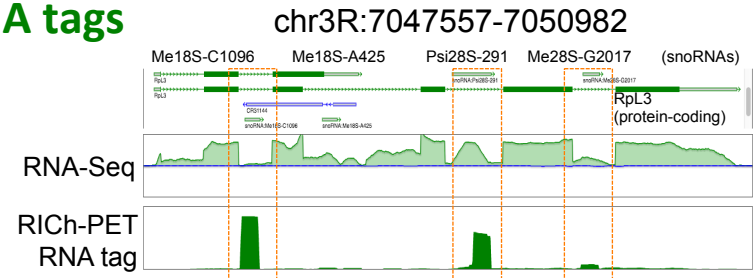Rinn and Guttman Science 2014

3D Nucleome

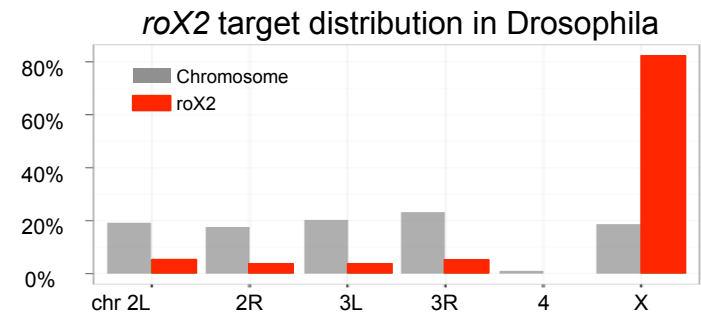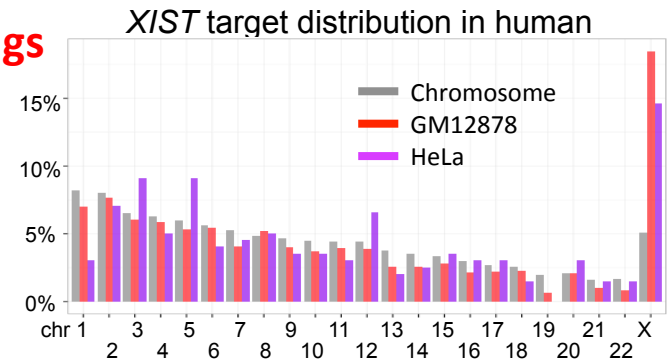# Genome-wide approach for RNA-chromatin interactions

RNA Interaction with Chromatin by Paired End Tag Sequencing, **RICh-PET**

A

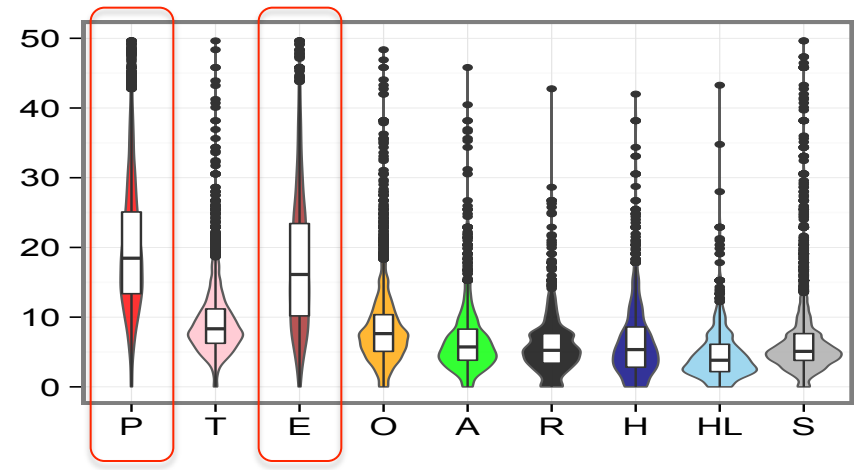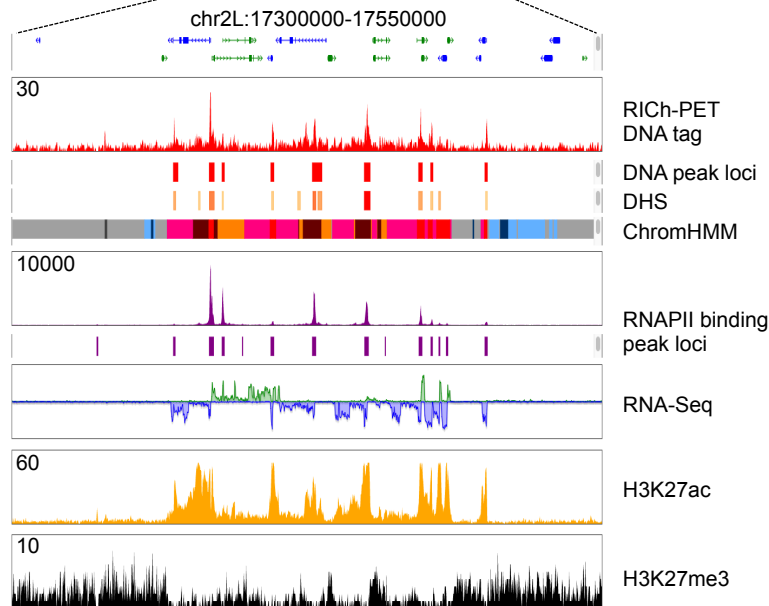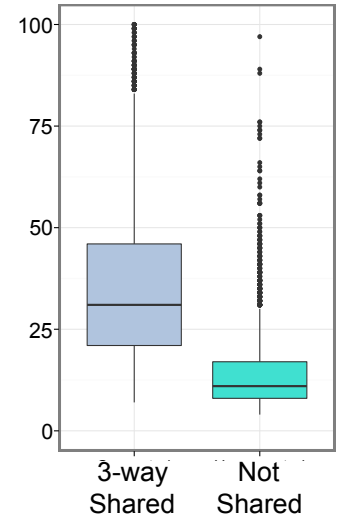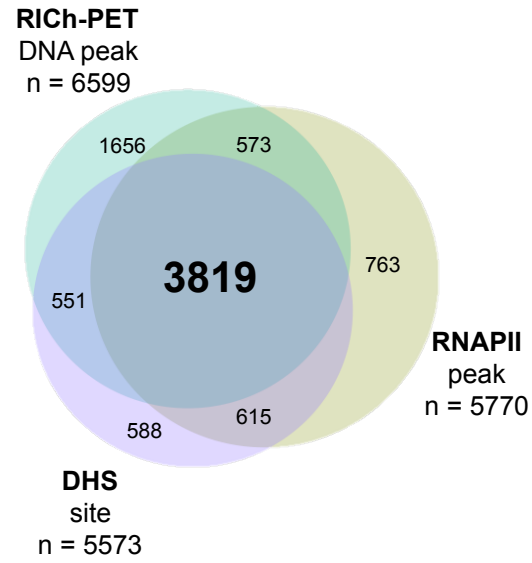**RNA-chromatin contacts**

Crosslinking fragmentation

Linker annealing cDNA synthesis DNA ligation

RNA linker
NNNNNN

DNA linker

RNA linker anneal
RT to make 1st cDNA

DNA linker ligation

NNNNNN

Proximity ligation

2nd strand cDNA

PET template Amplification Sequencing

PET mapping

Intra-chromosome

Inter-chromosome

RNA tags chr3R:7047557-7050982

Me18S-C1096   Me18S-A425   Psi28S-291   Me28S-G2017   (snoRNAs)

RpL3

CRS144

snoRNA.Me18S-C1096   snoRNA.Me18S-A425   snoRNA.Me28S-G2017

snoRNA.Psi28S-291

RpL3
(protein-coding)

RNA-Seq

RICh-PET
RNA tag

DNA tags

XIST target distribution in human

15%

10%

5%

0%

chr 1   3   5   7   9   11   13   15   17   19   21   X
      2   4   6   8   10   12   14   16   18   20   22

Chromosome
GM12878
HeLa

roX2 target distribution in Drosophila

80%

60%

40%

20%

0%

chr 2L   2R   3L   3R   4   X

Chromosome
roX2

Meizhen Zheng, Oscar Luo
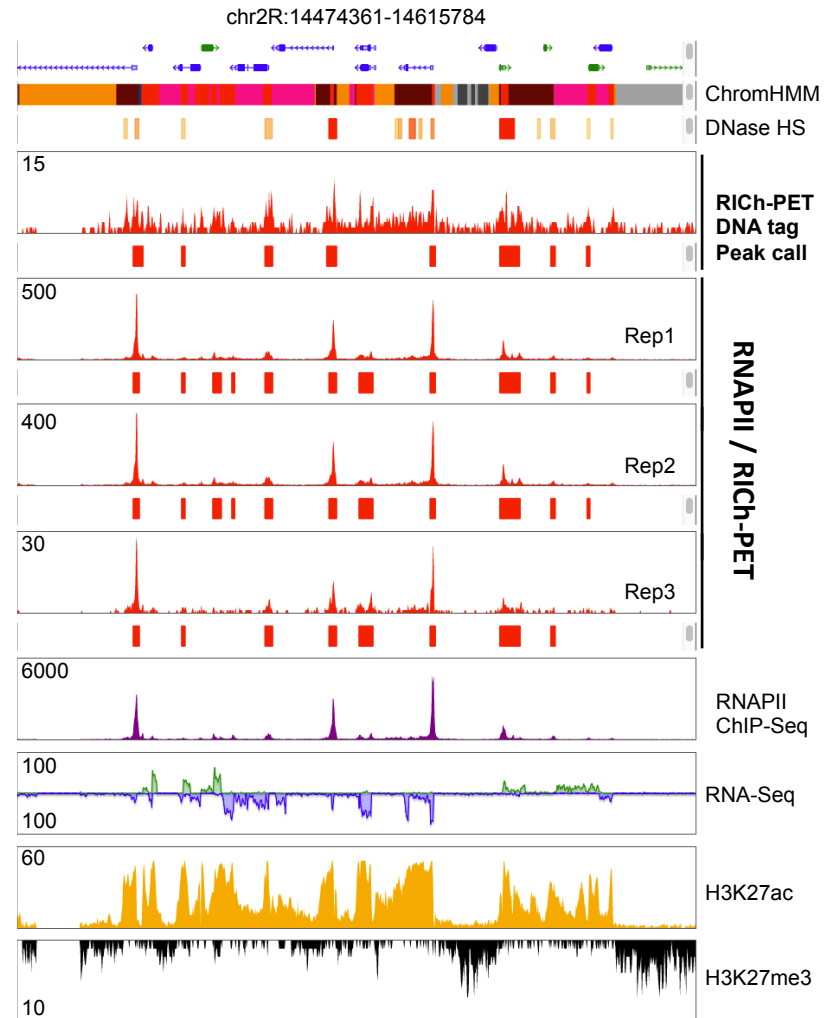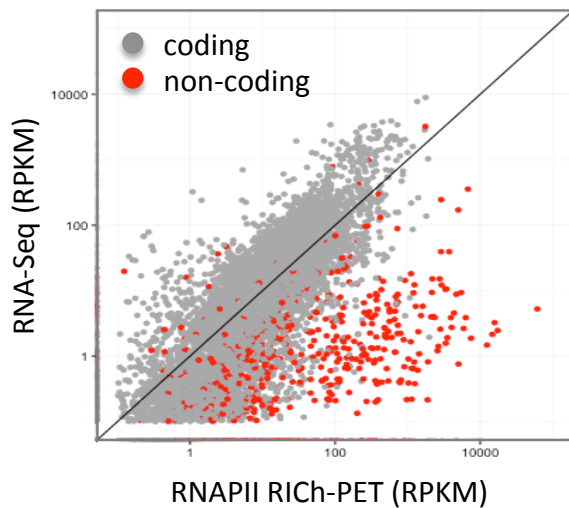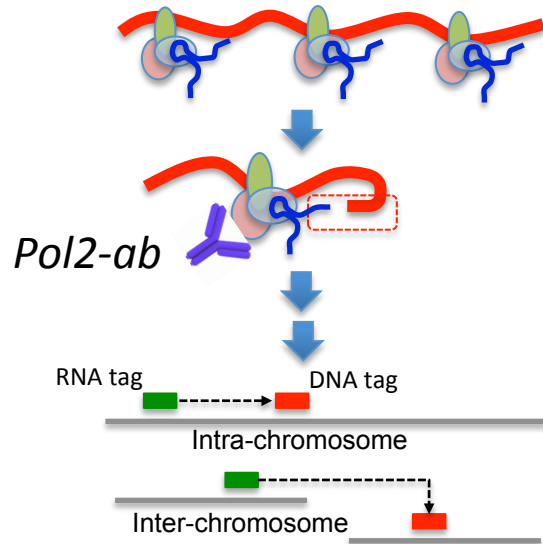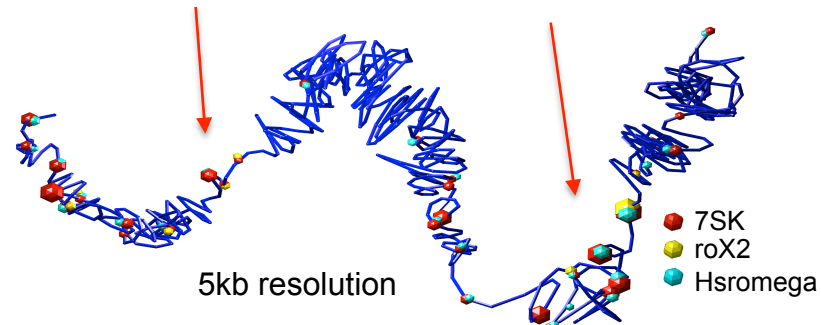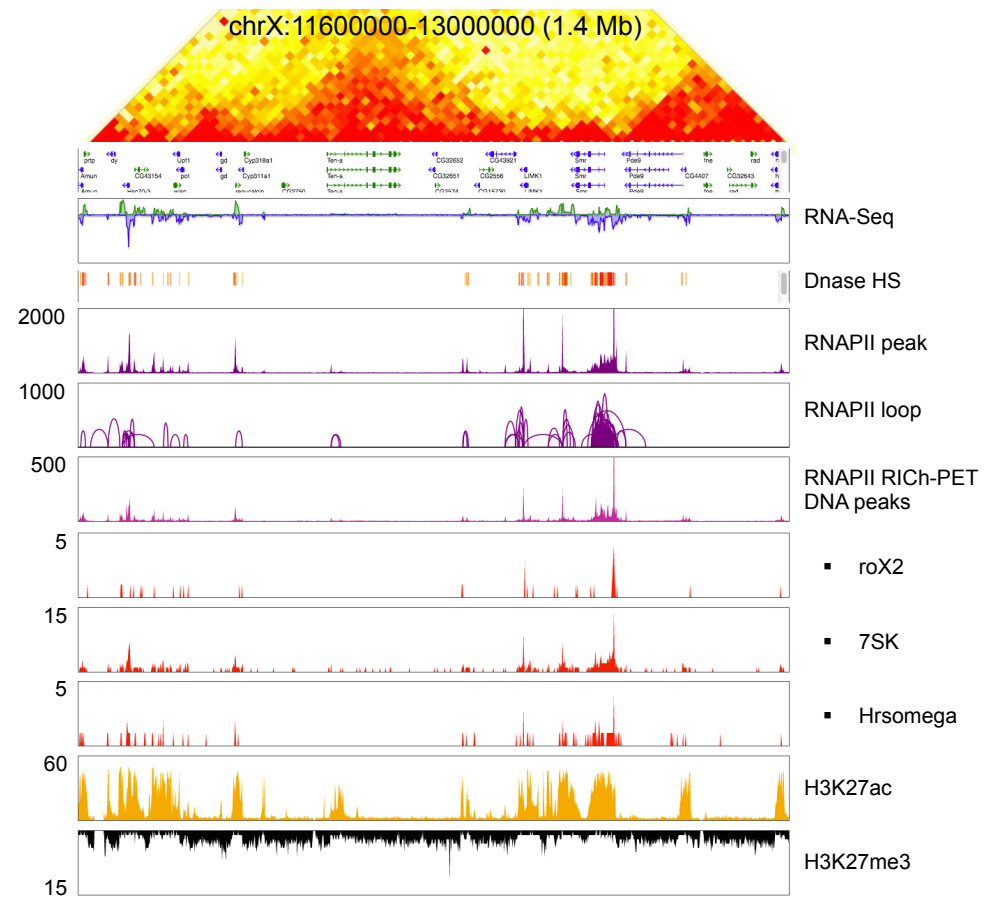
Most ncRNAs target active open chromatin loci
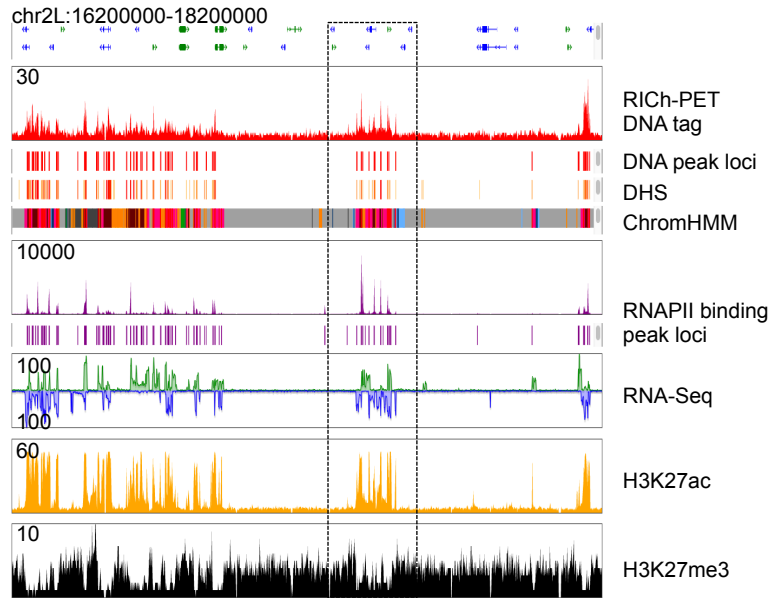
> **Enriched in promoter & enhancer regions**

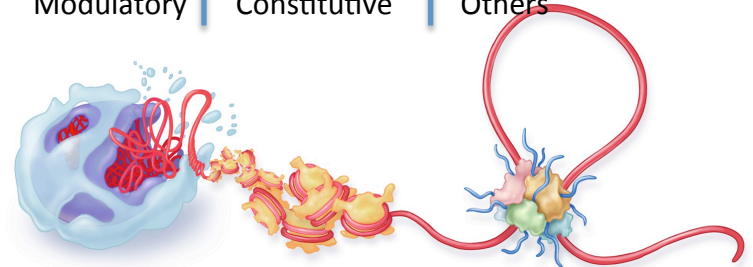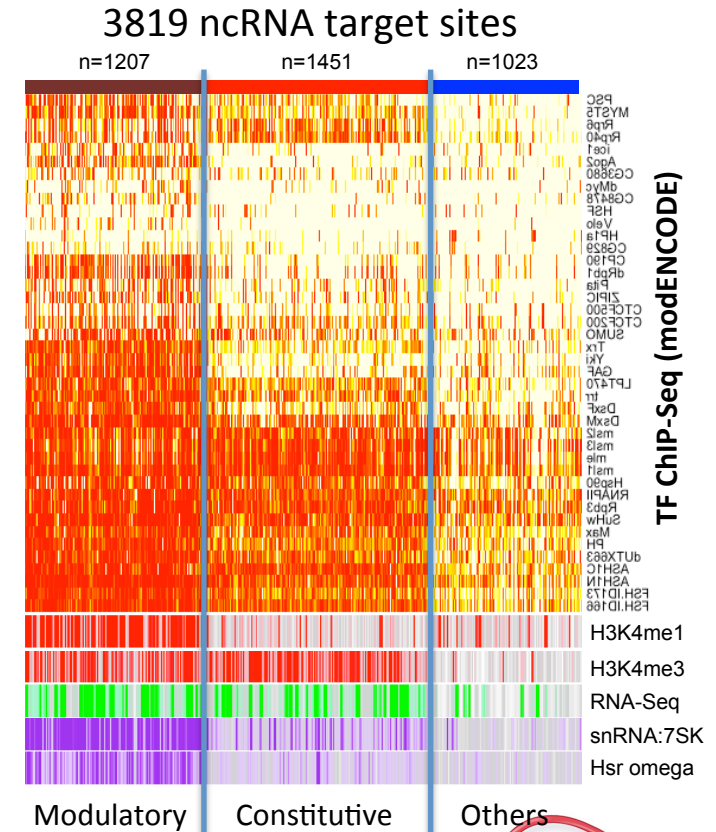# RNAPII ChIP / RICh-PET for detecting transcriptional RNA-chromatin interactions

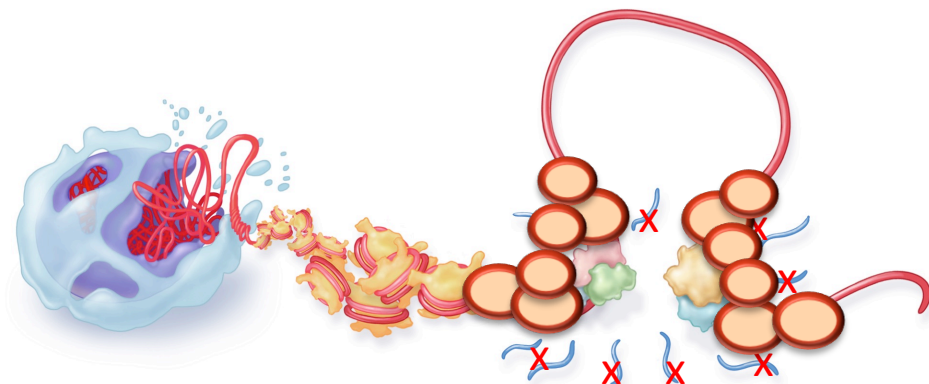# ncRNA contacts enriched at TAD boundary regions

Combinatory binding by multiplex protein and RNA factors

chr3R:26198479-26310882

RNA-seq

DHS

RNAPII RICh-PET
DNA tag

7SK

Hsr omega

RNA factors

Protein factors (modENCODE)

H3K27ac

H3K27me3

3819 ncRNA target sites

n=1207   n=1451   n=1023

TF ChIP-Seq (modENCODE)

H3K4me1
H3K4me3
RNA-Seq
snRNA:7SK
Hsr omega

Modulatory   Constitutive   Others
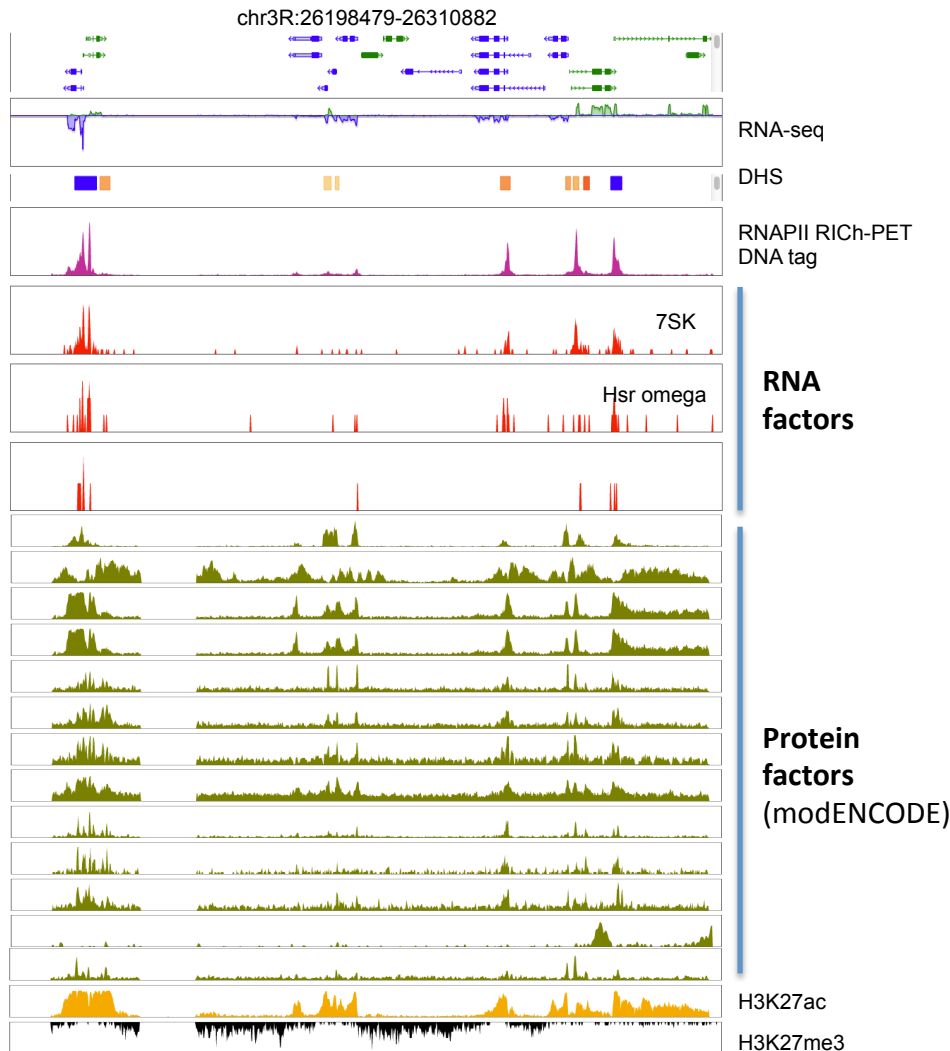
To test the role of ncRNAs, …

Immuno-stain

RNase A -   RNase A +

DAPI

RNAPII

Merge

2 μM   3 μM

ATAC-Seq

RNaseA+
(100µg/mL)

(200µg/mL)

(400µg/mL)

RNaseA-
control

RNAPII ChIA-PET

chr2L:13196092-13314472

RNA-seq

Chromatin state

DHS

ATAC-seq

20   Control, RNaseA-

20   100µg/mL RNaseA

20   200µg/mL RNaseA

20   400µg/mL RNaseA

RNAPII-loop

15   PET=14   Control, RNaseA-

15   PET=6   200µg/mL RNAseA

15   PET=6   400µg/mL RNaseA
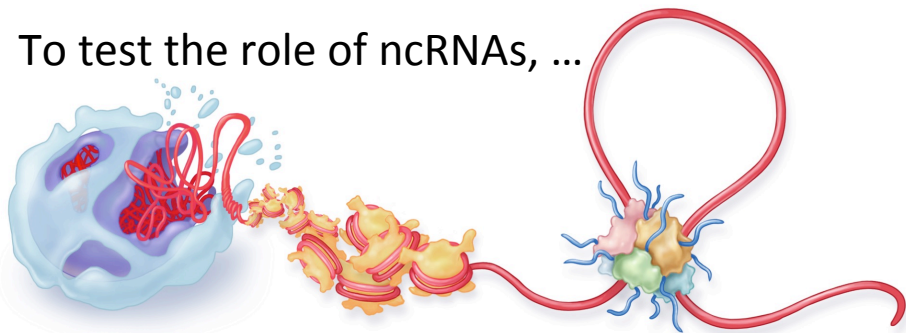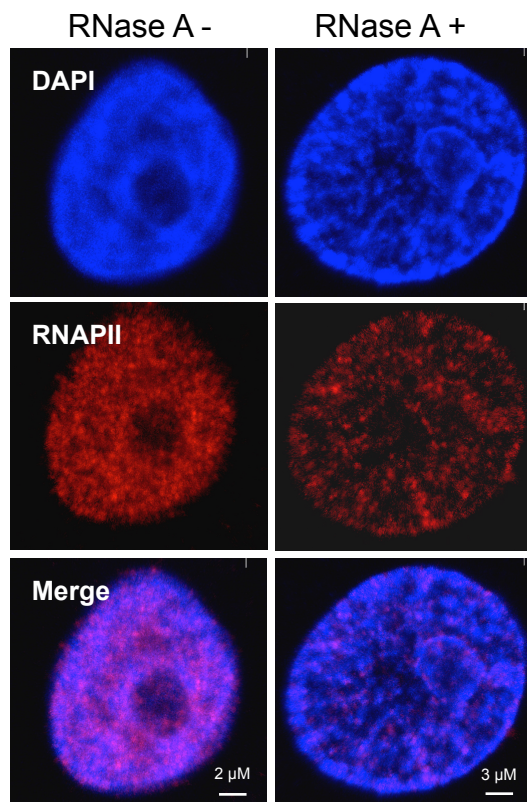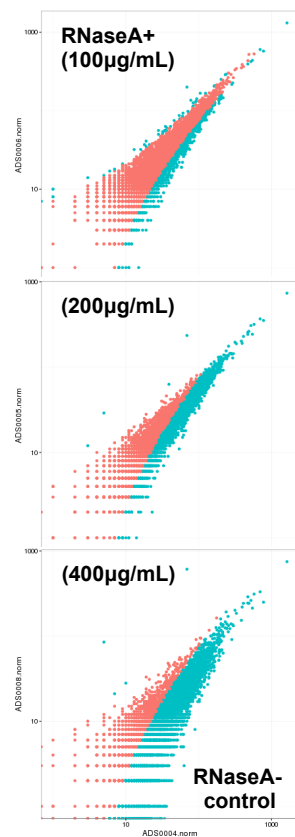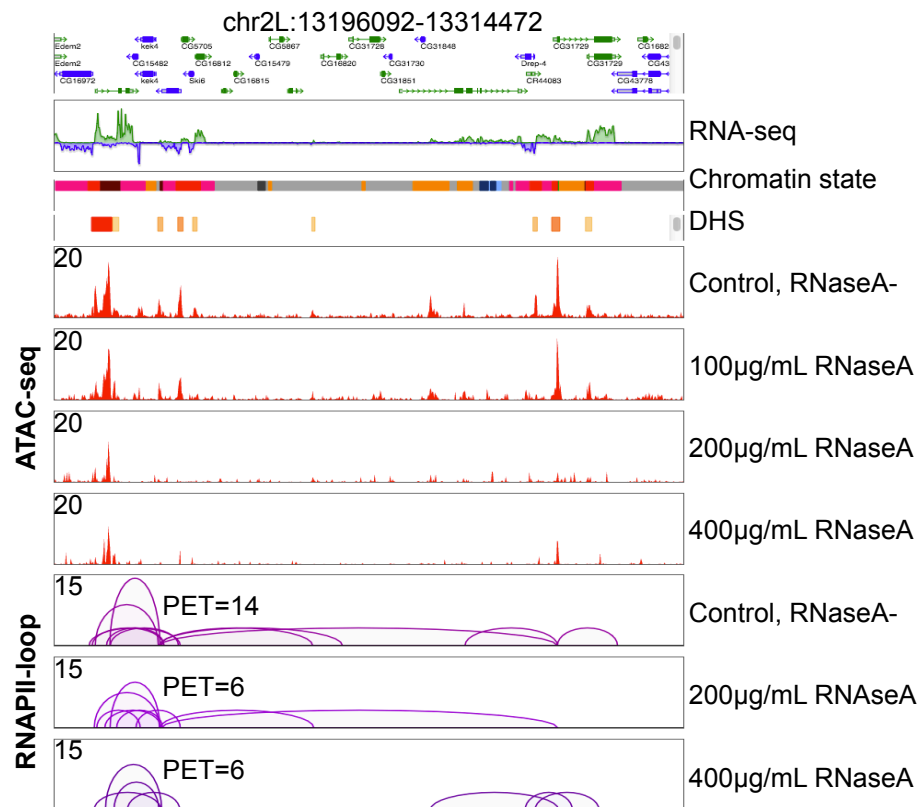
Acknowledgements

Lab members

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Jufen Zhu
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Przemek Szałaj
Grzegorz M. Wilczynski

MNHN, Paris
Laurent Sachs

Roux Family