

# WORKSHOP

## Transcriptional circuitry and the regulatory conformation of the genome

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Faculty of Life Sciences

# Chromosome conformation capture (3C)

# Most GR Binding Sites Are Distant From Regulated Genes

Transcription  
Pol II ChIP

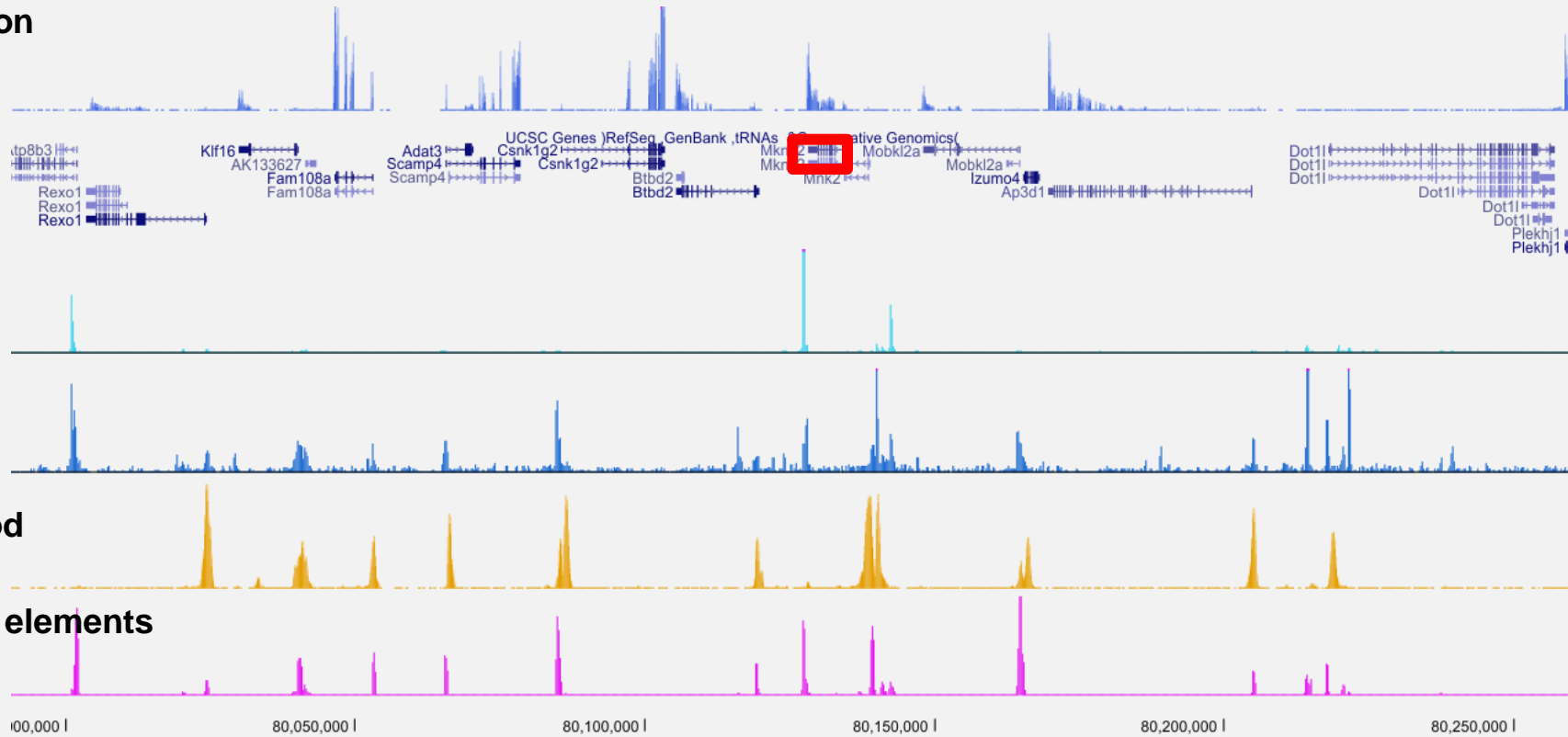
Genes

TF1  
GR

TF2  
AP1

Histone mod  
H3K4Me2

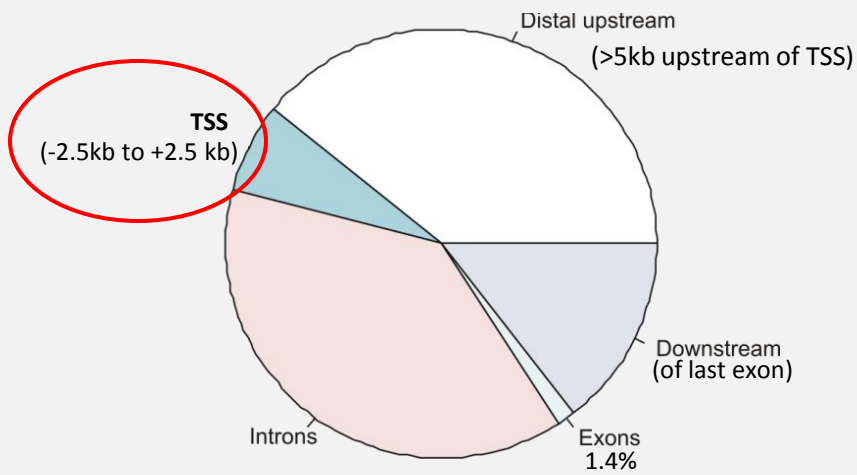
Regulatory elements  
DHS



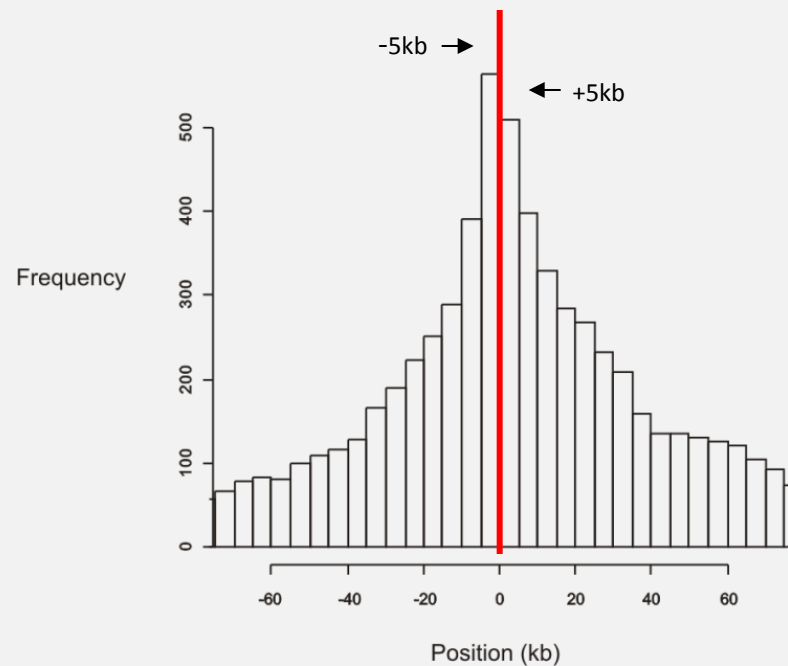
chr10:79,970,001-80,260,000

# Distribution of GR Binding Sites

## 8,373 GR Binding Sites



## Position of GR relative to TSS

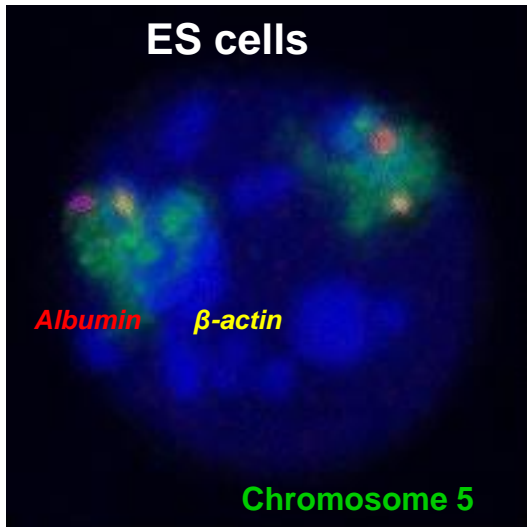


# The genome is not randomly organized



Nature Reviews Genetics 6, 429

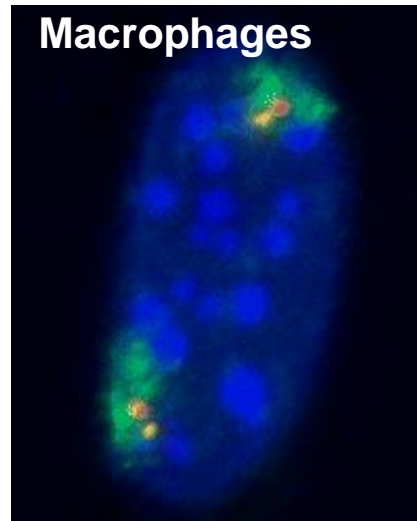
Chromosomes territories



ES cells

*Albumin* *β-actin*

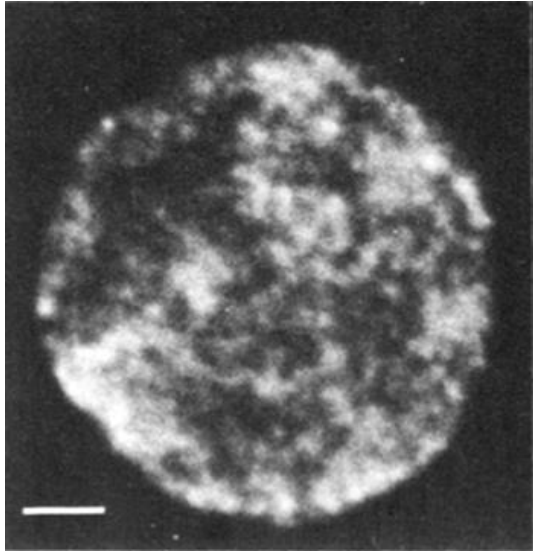
Chromosome 5



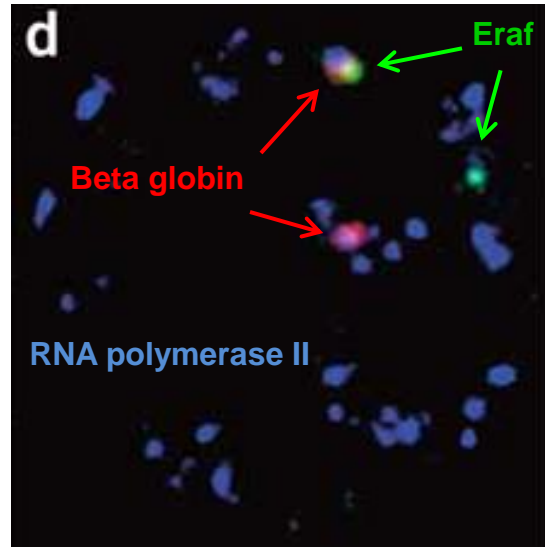
Macrophages

Cell-type specificity

# Functional compartmentalization

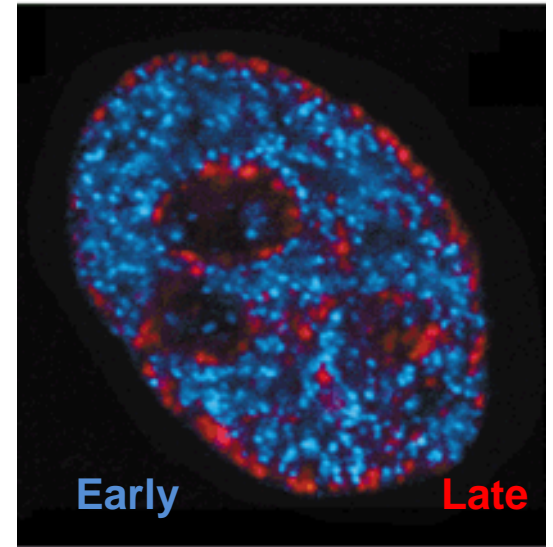


**Active chromatin  
DNase I**

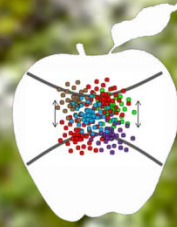


**Expression foci**

**Gene position is  
correlated with gene activity**



**Replication timing**

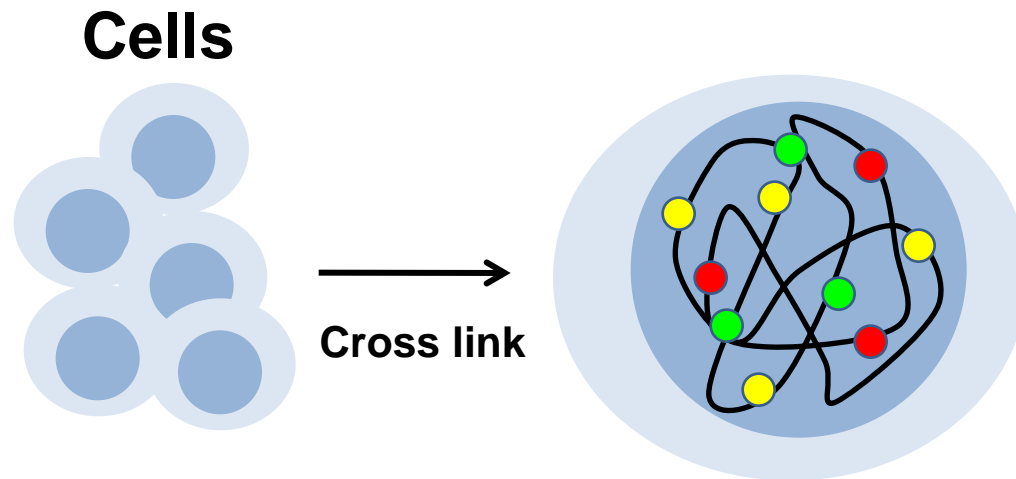


- **Increase resolution**
- **Increase throughput**
- **Reduce bias**



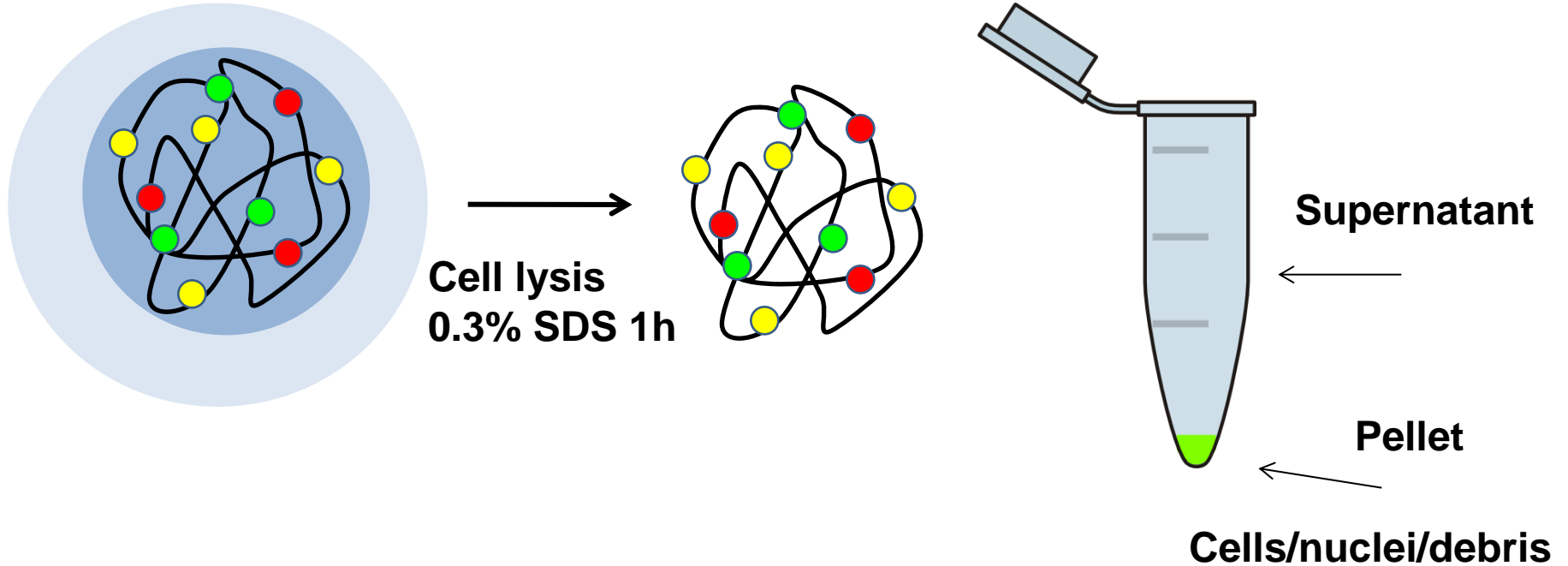
# Chromosome Conformation Capture (3C)

## 1. Crosslinking With Formaldehyde



**FA%-0%-2% we commonly use 1%**  
**Time- commonly 10 minutes**  
**Temperature- commonly 37° C**

## 2. lysis?



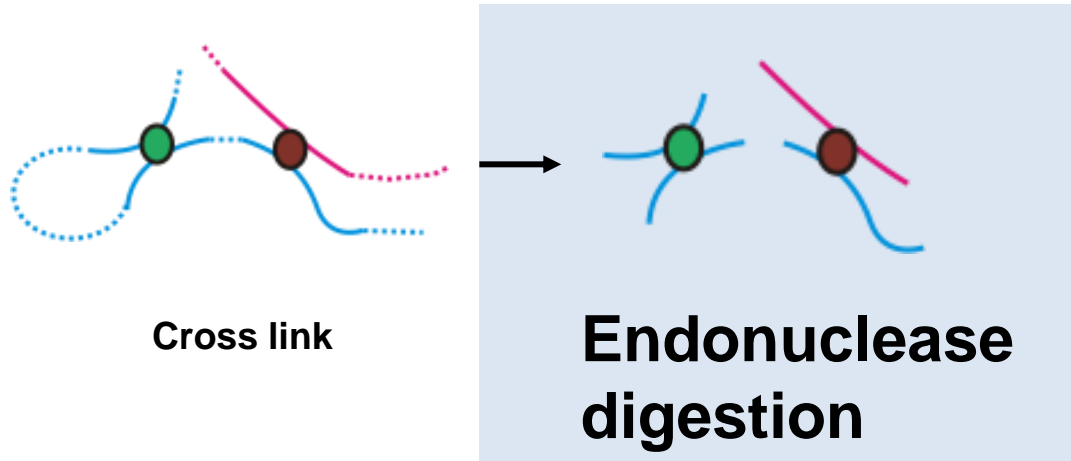
***In vivo* formaldehyde cross-linking: it is time for black box analysis**

**Gavrilov et al., PLoS One. 2013;8(3):e60403**

**Gavrilov et al., Brief Funct Genomics, in press**

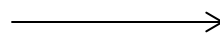


# 3. Endonuclease digestion



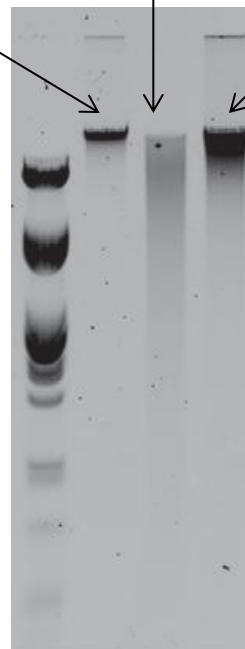
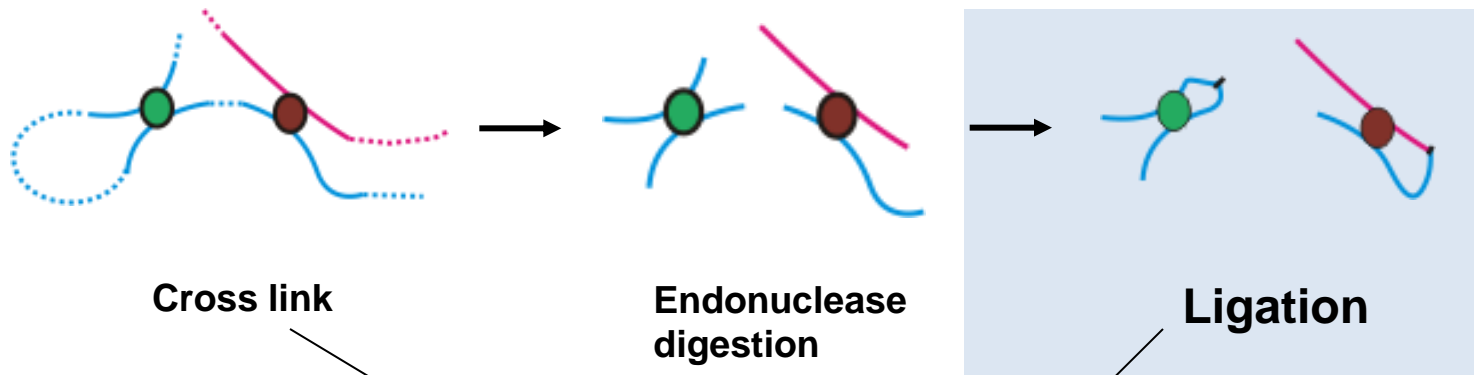
**Search the literature for validated enzymes capable digesting in 3C conditions (SDS, Triton...)**

**6 b.p. cutter  
4 b.p. cutter**



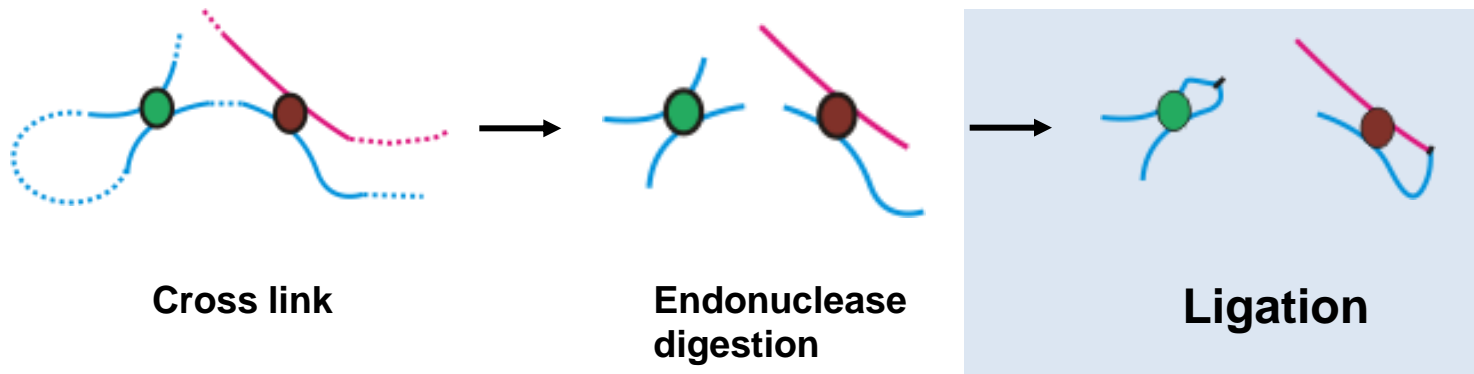
**We will discuss resolution issues**

# 4. Ligation



**Quality control**

# 4. Ligation

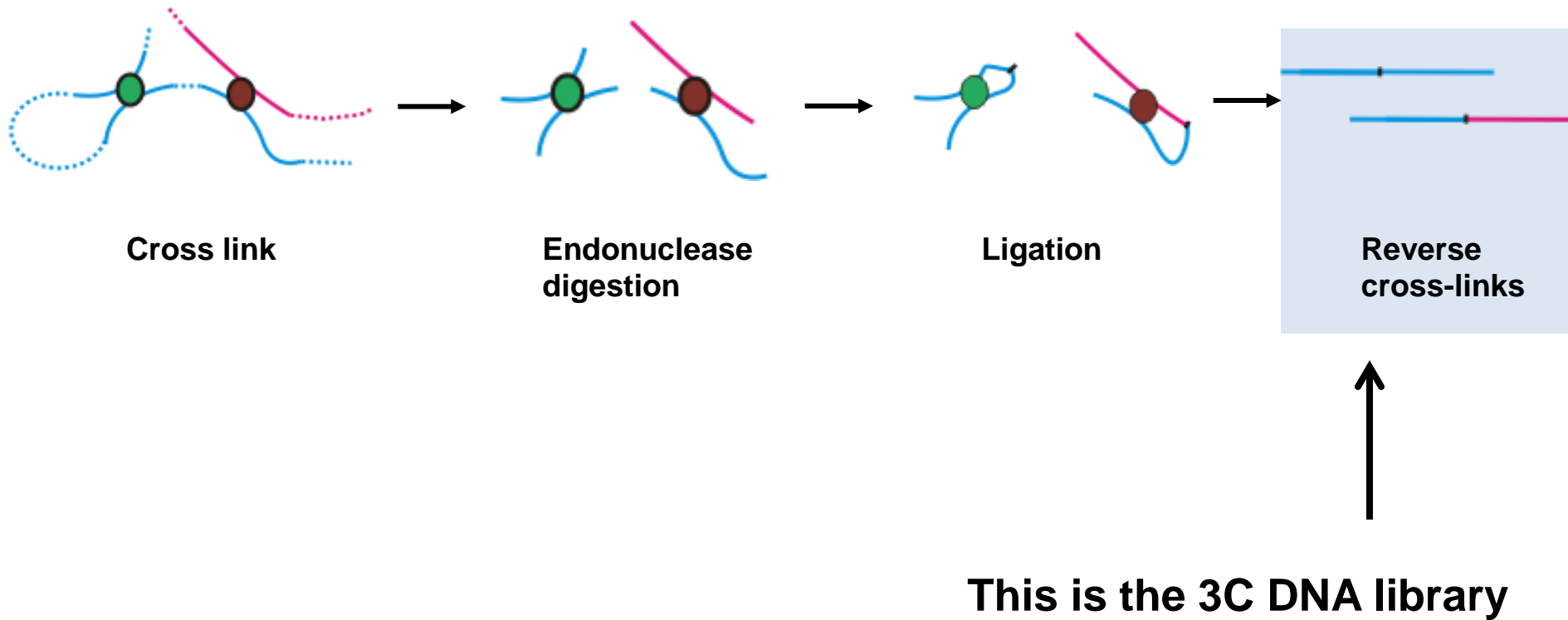


**Low DTT in ligation buffer is important for the following DNA precipitation**

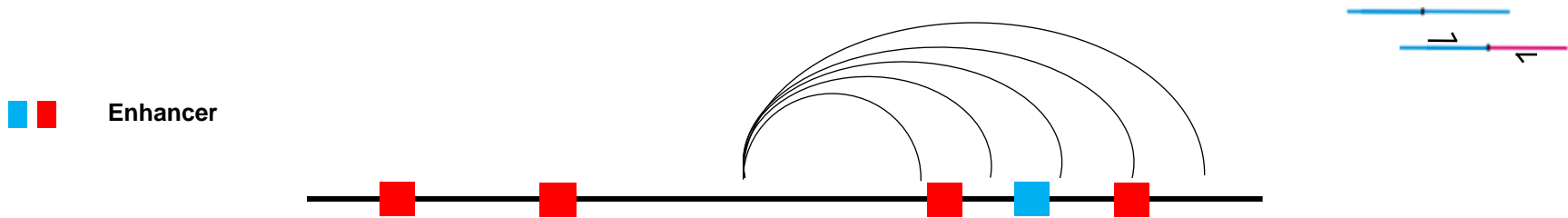


**Multiple ligases can work in low DTT concentrations suitable for 3C**

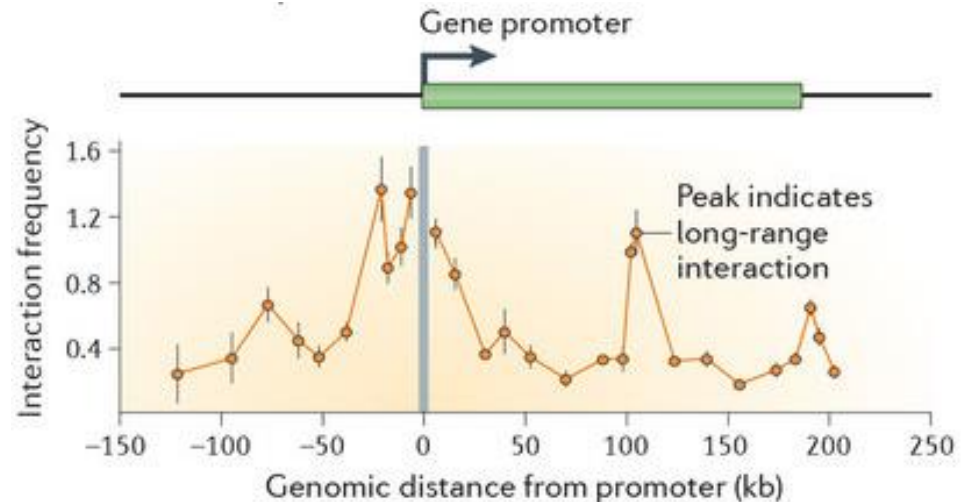
# 5. Reverse cross-links



# Chromosome Conformation Capture (3C)

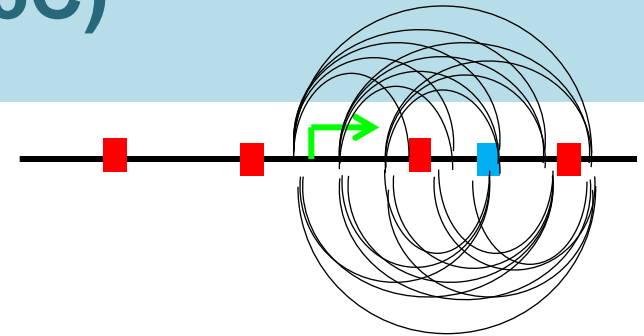


Principle	Contacts between two defined regions
Coverage	Commonly <1Mb
Detection	Locus-specific PCR
Resolution	High
Limitations	Low throughput and coverage
Examples	Determine interaction between a known promoter and enhancer



# Carbon Copy 3C (5C)

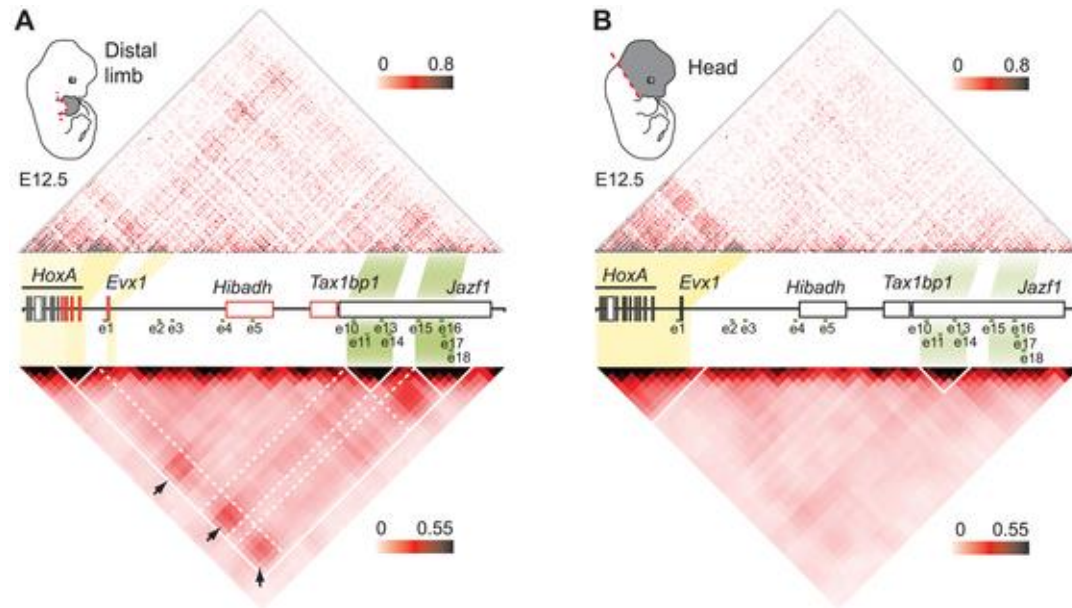
Principle	All against all
Coverage	Commonly <1Mb
Detection	Multiplex PCR, HT-sequencing
Resolution	High
Limitations	Limited coverage
Examples	Determine comprehensively higher-order chromosome structure in a defined region



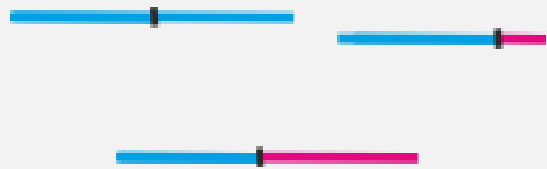
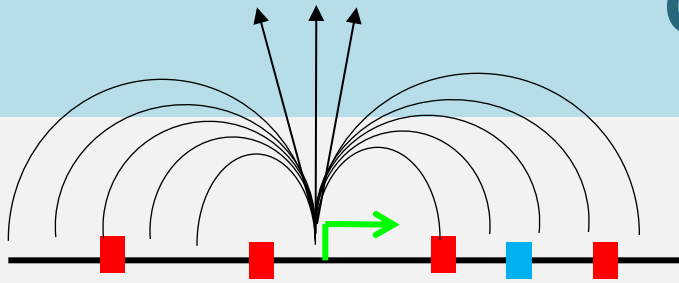
## Multiplex PCR



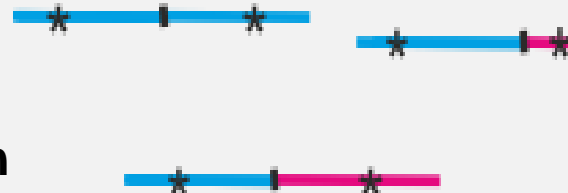
## Limb-specific contacts



# Circular 3C (4C)

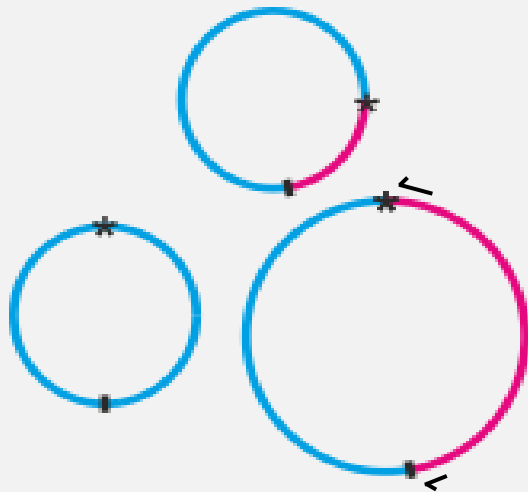


Second digestion



3C DNA

Ligation

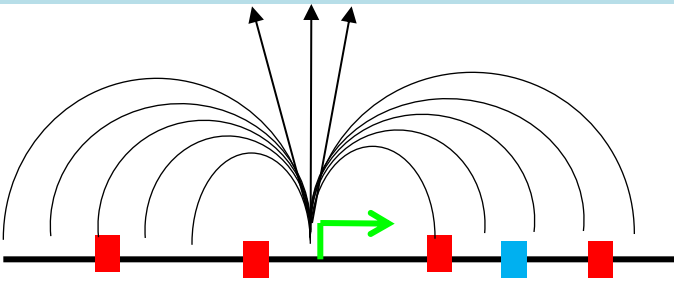


PCR



Sequencing

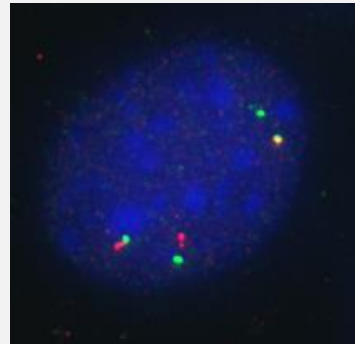
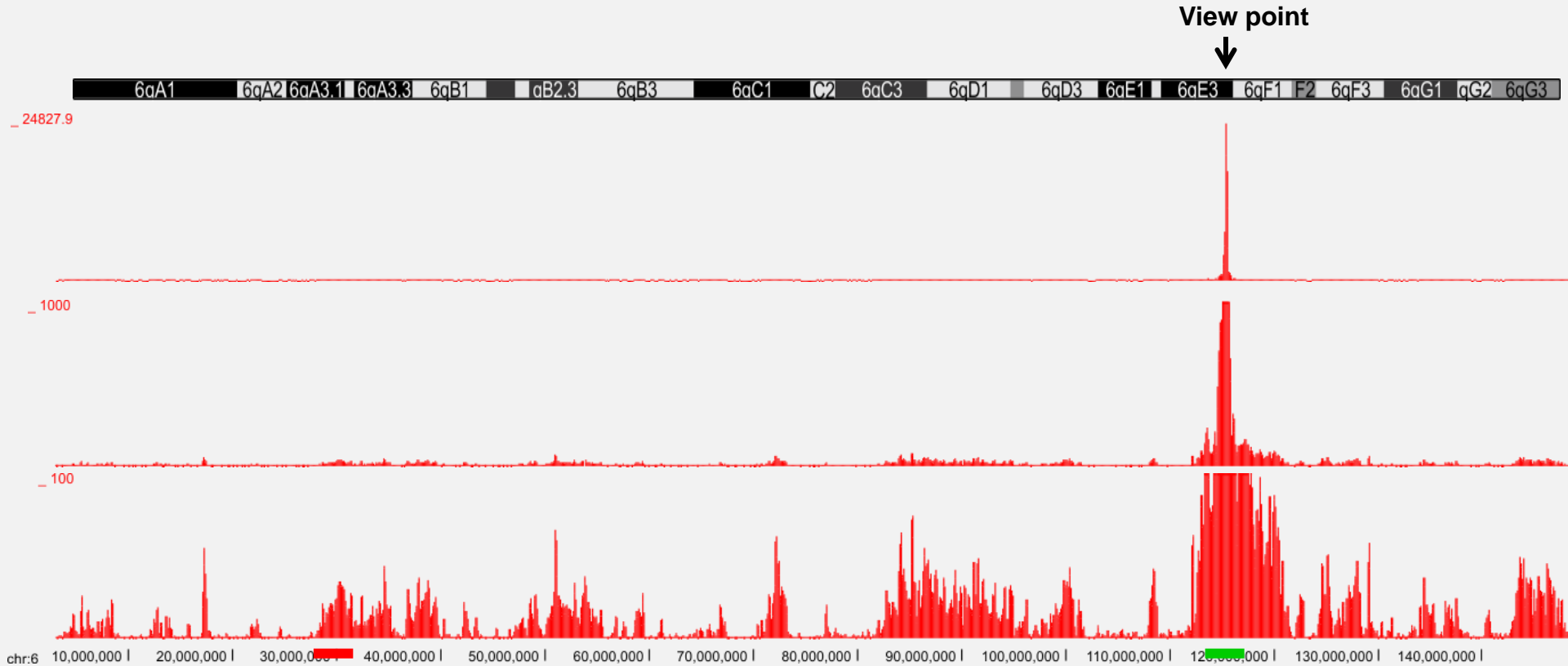
# Circular 3C (4C)



Principle	All contacts with a point of interest
Coverage	Genome-wide
Detection	PCR, HT-sequencing
Resolution	High
Limitations	Limited to one view point
Examples	All genes and genomic elements associated with a known LCR



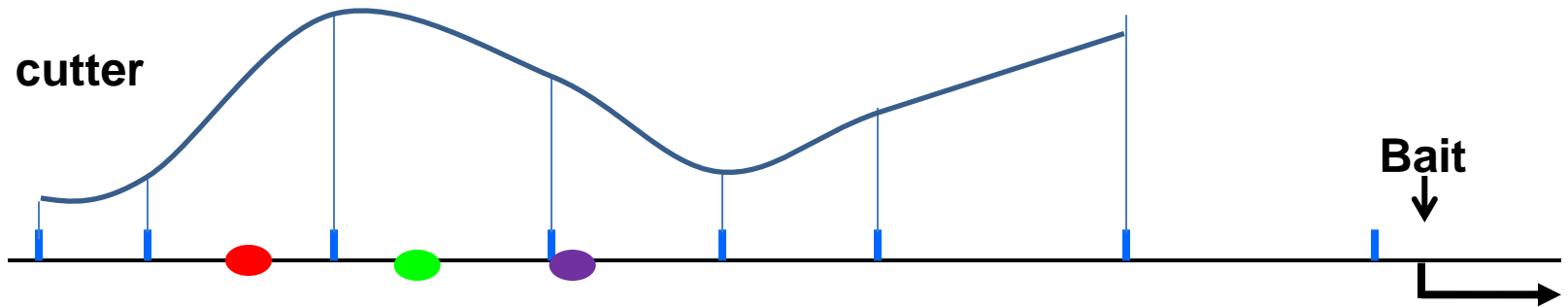
# Hierarchical Contact Landscape



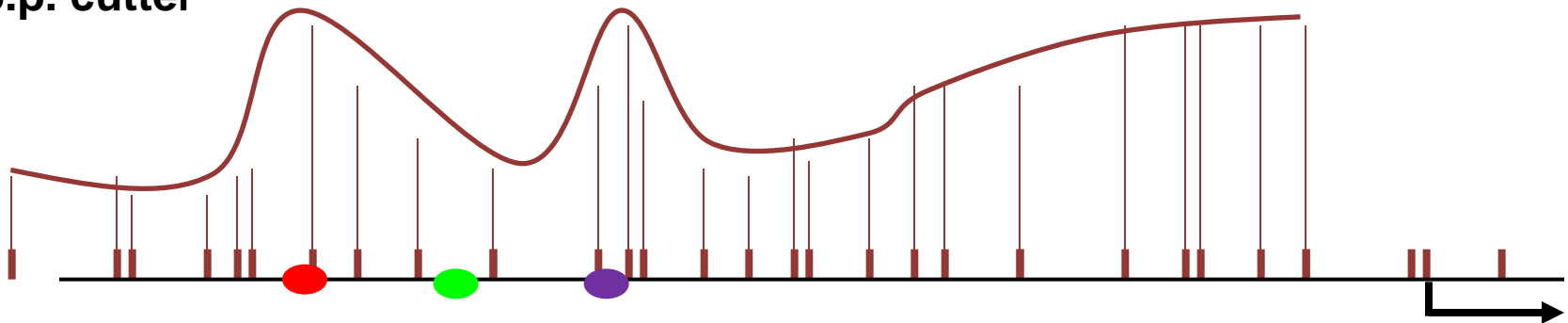
# Resolution - proximal range 10kb- 1000kb

High contact frequency

6b.p. cutter



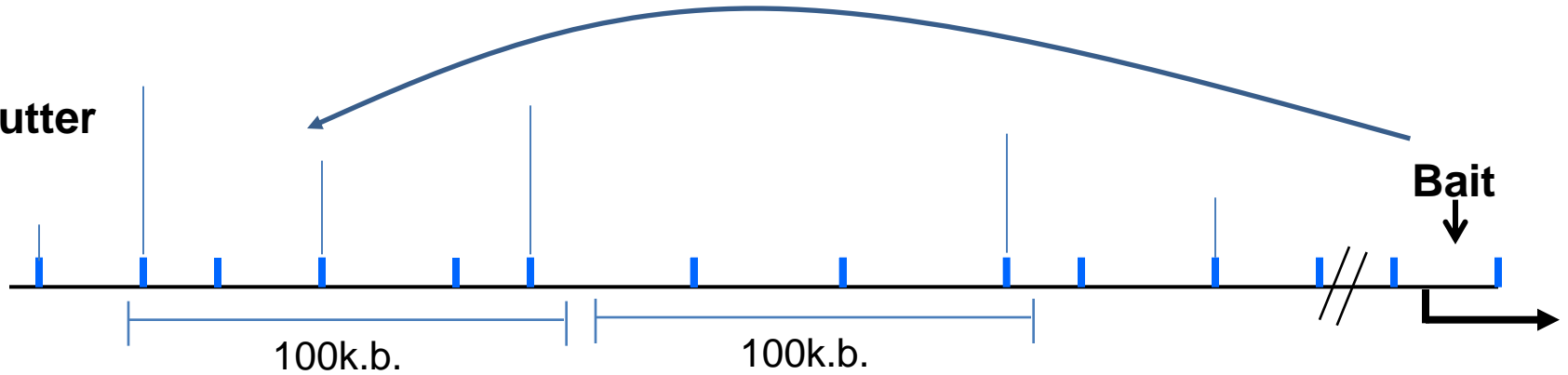
4b.p. cutter



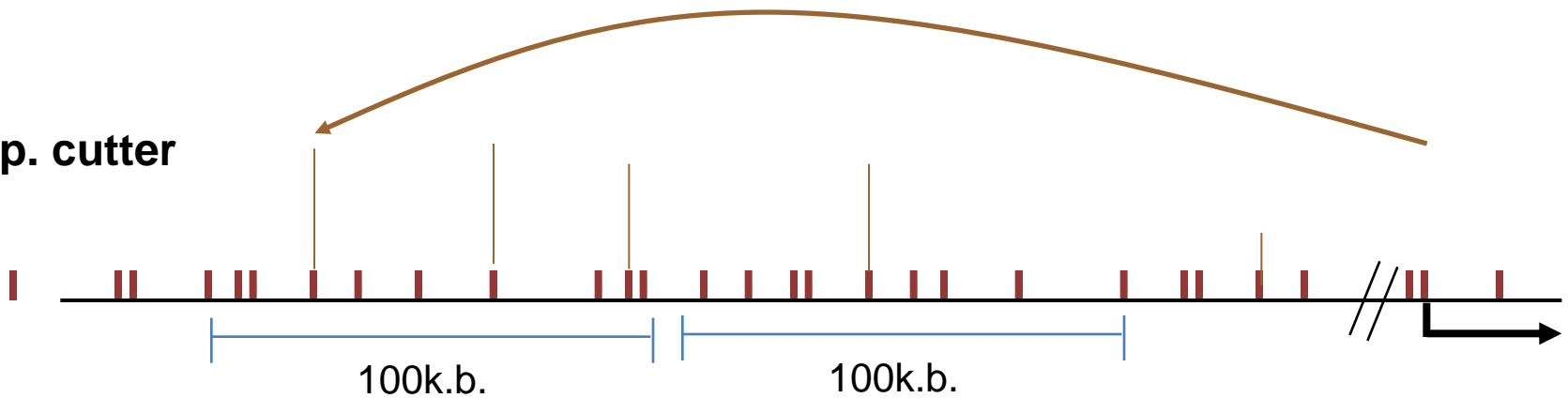
# Resolution - far cis (Mb) and trans

Low contact frequency

6b.p. cutter

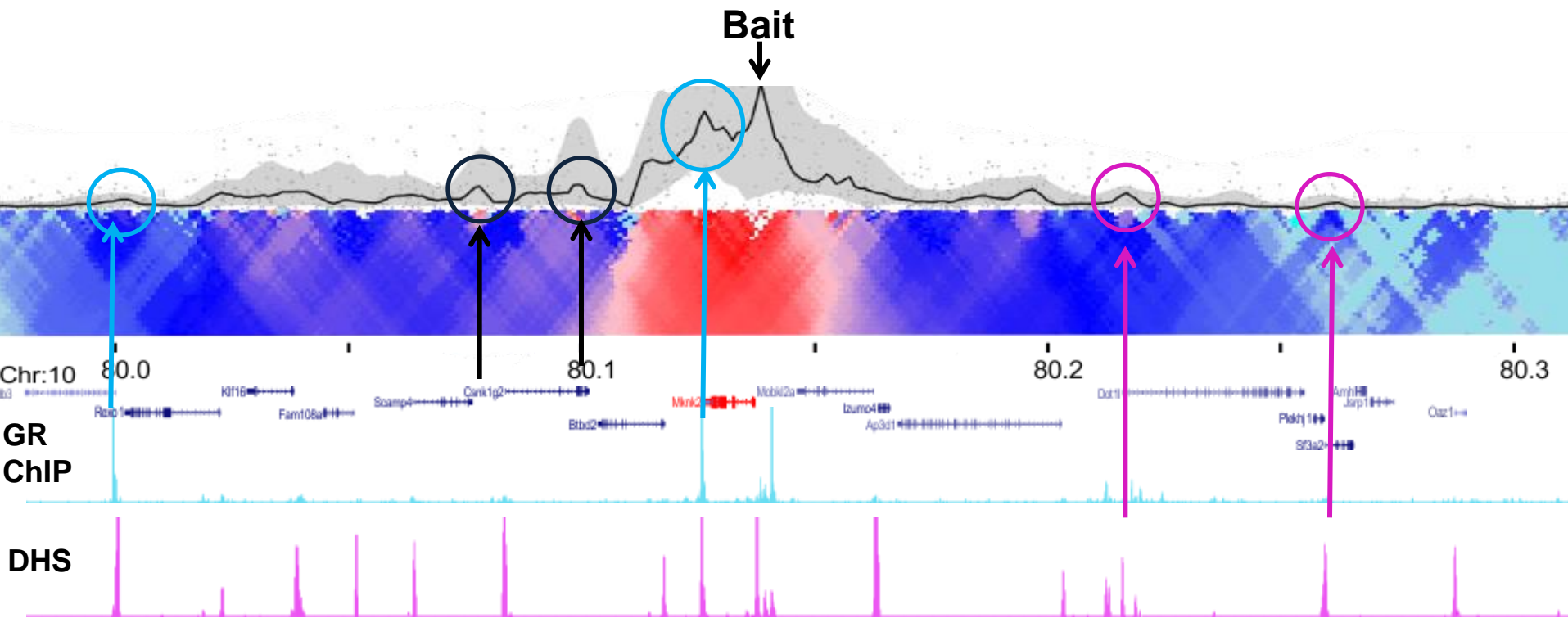


4b.p. cutter



# *Mknk2* Long-Range Contacts High-Resolution

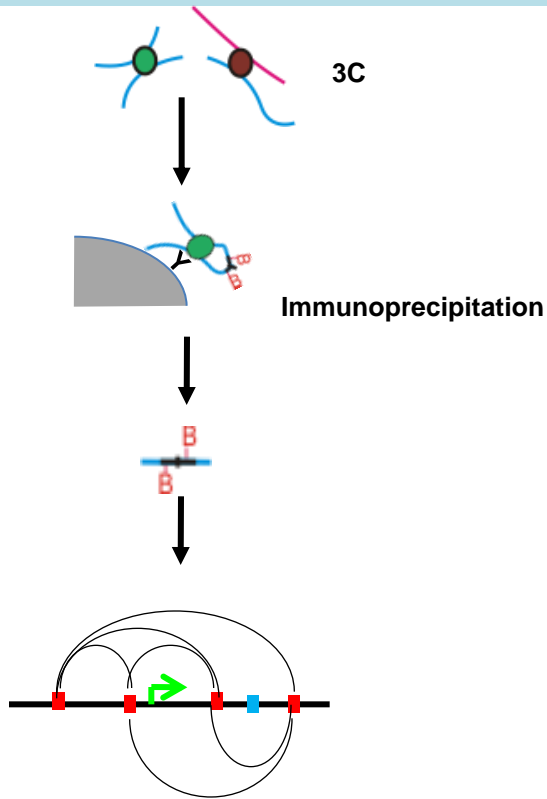
Contacts 1h after induction



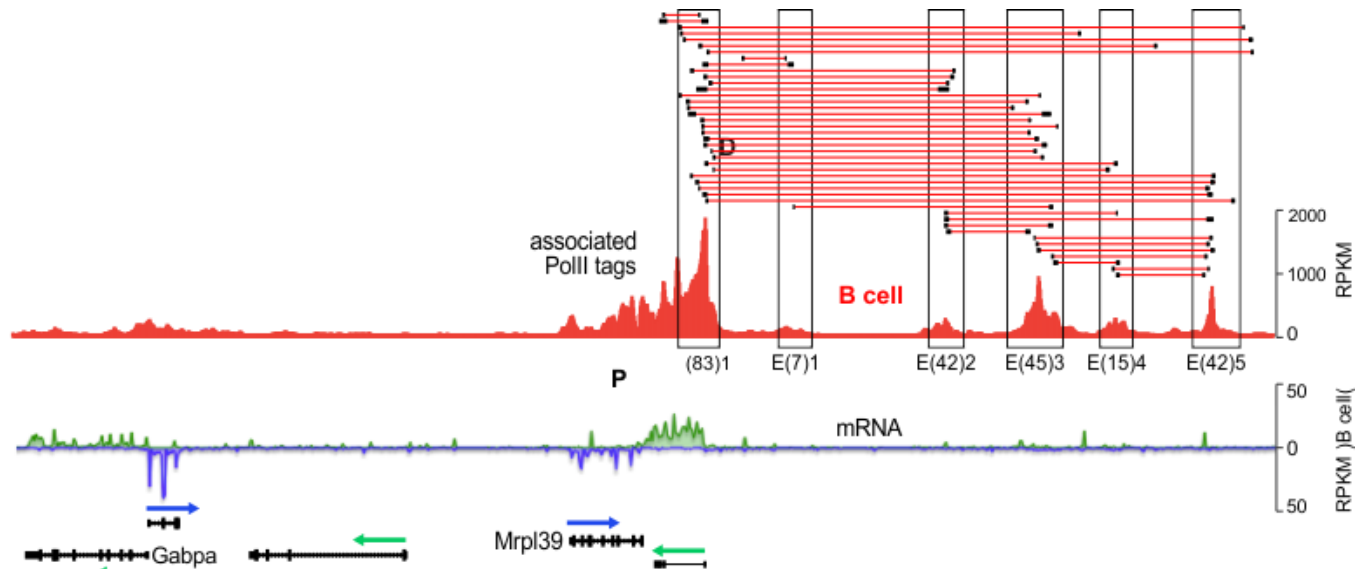
Rachel Deitch, Dana Raz, Moran Tal

Analysis method- van de Werken et al., Nat. Methods 2012

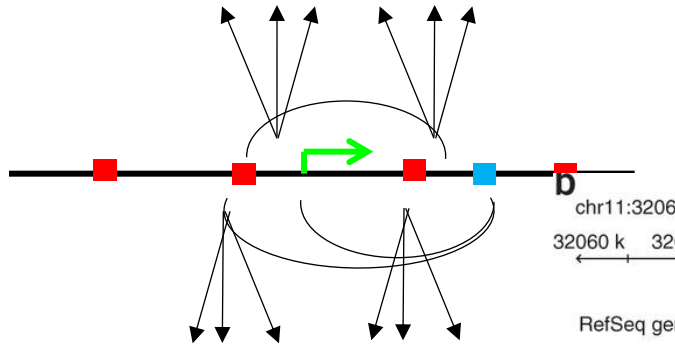
# ChIA-PET



Principle	All contacts associated with a given protein
Coverage	Genome wide *
Detection	paired end HT-sequencing
Resolution	High
Limitations	Rely on one factor, disregarding other contacts
Examples	Map chromatin interaction network of a known transcription factor

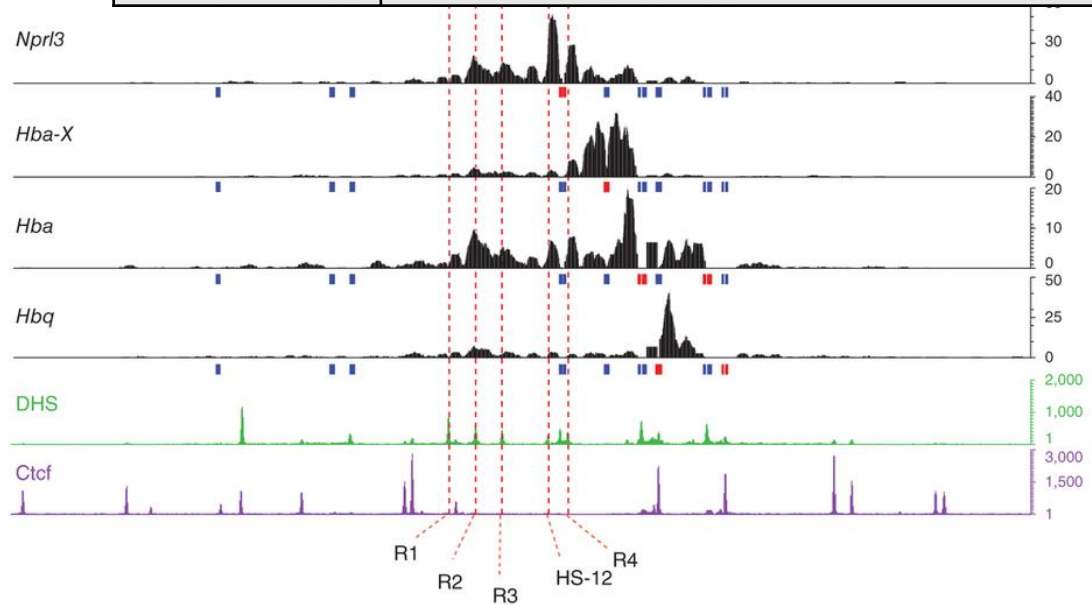


# Capture-C

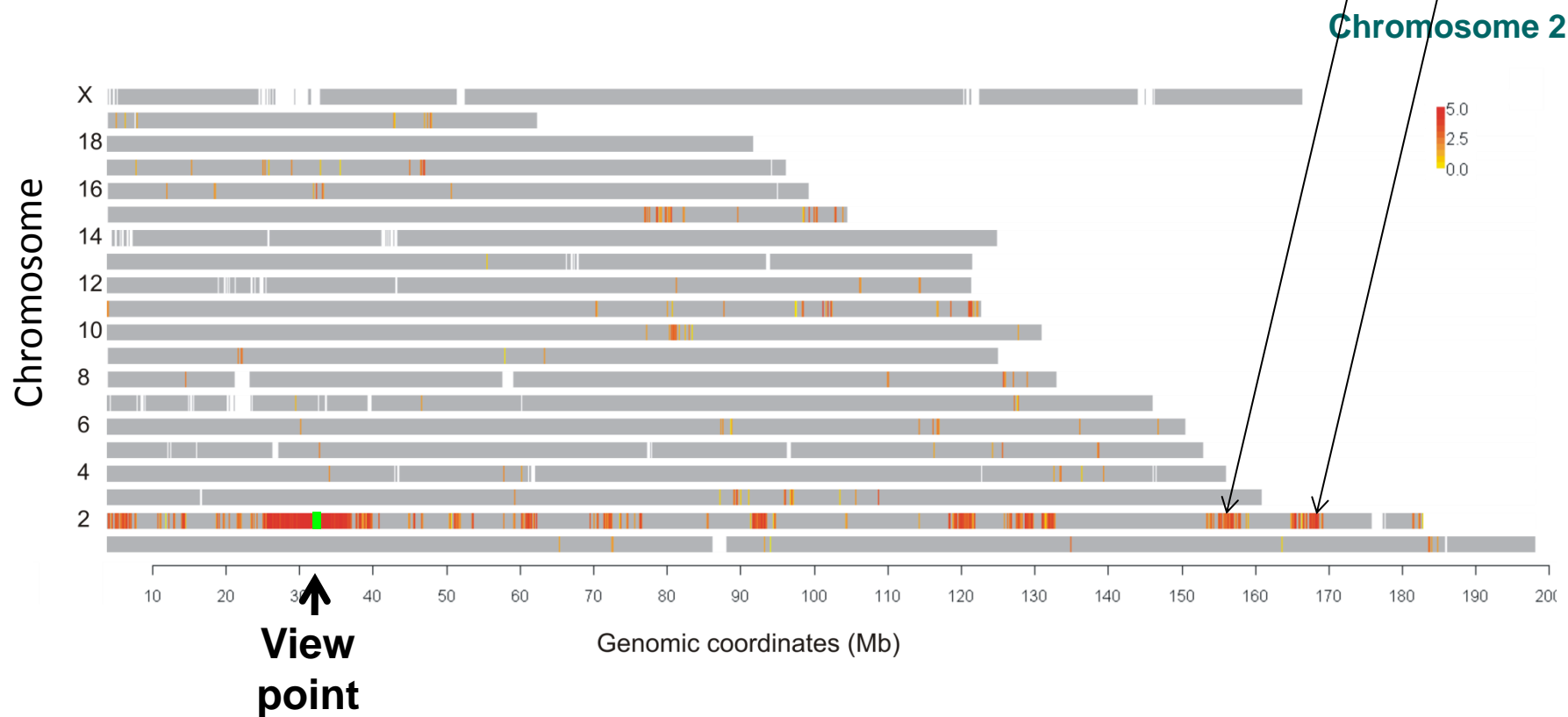
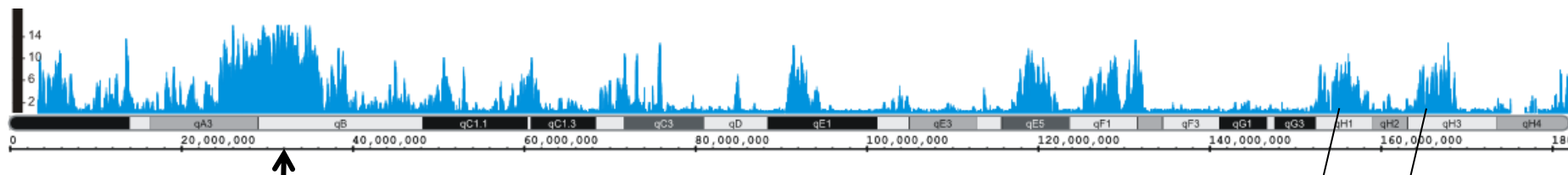
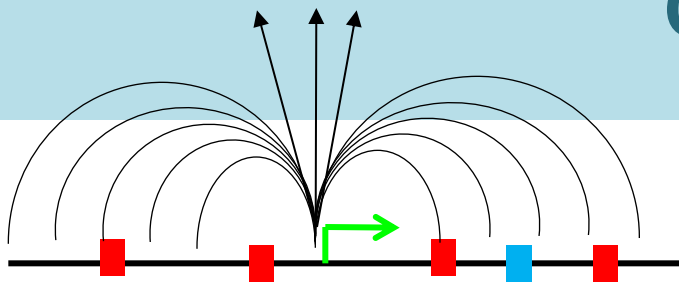


**Sub-sampling of Hi-C**

Principle	Focused all against all
Coverage	Up to genome-wide
Detection	HT-sequencing
Resolution	High
Limitations	Limited coverage
Examples	Determine comprehensively higher-order chromosome structure in multiple defined regions

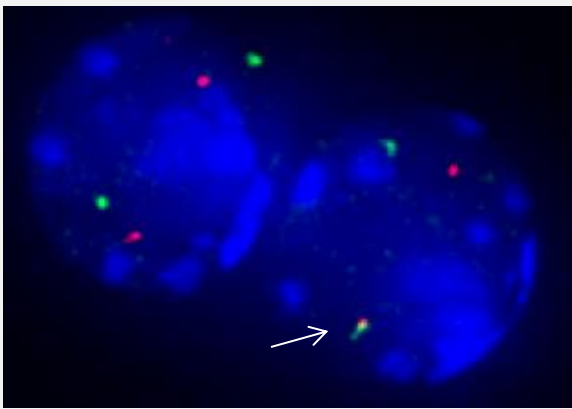


# Circular 3C (4C)

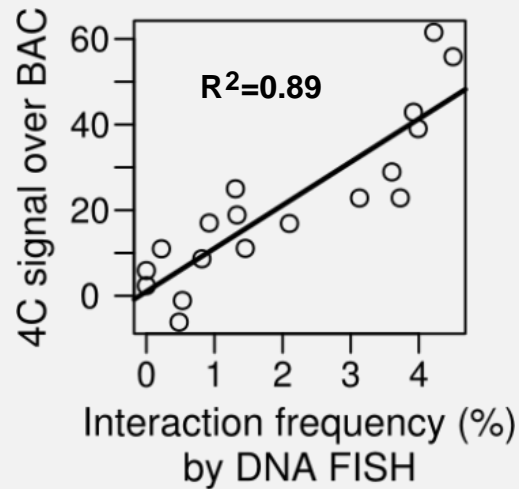


# What does a C signal mean?

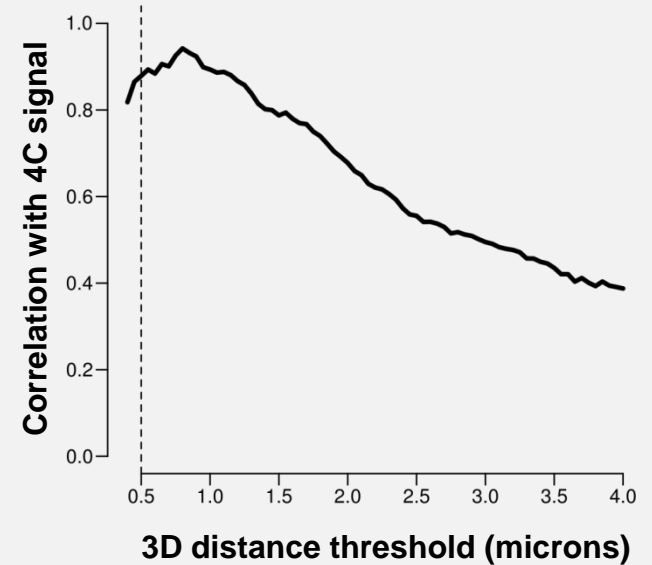
## DNA FISH



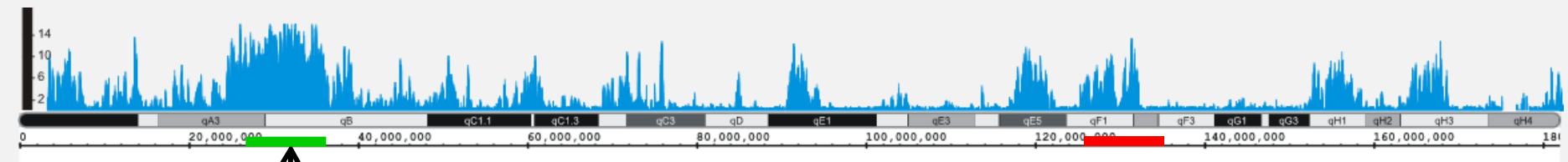
200-500 nuclei



$d < 0.5$  micron



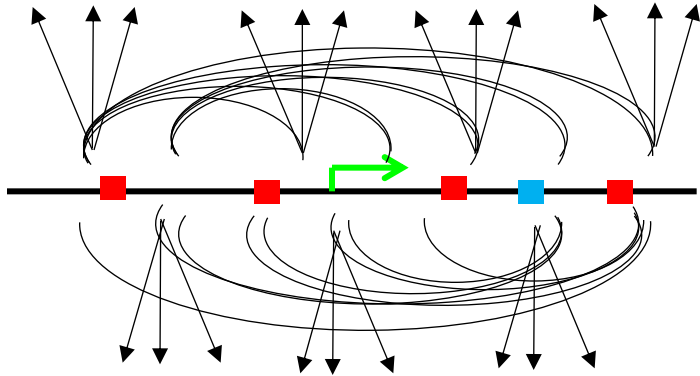
3D distance threshold (microns)



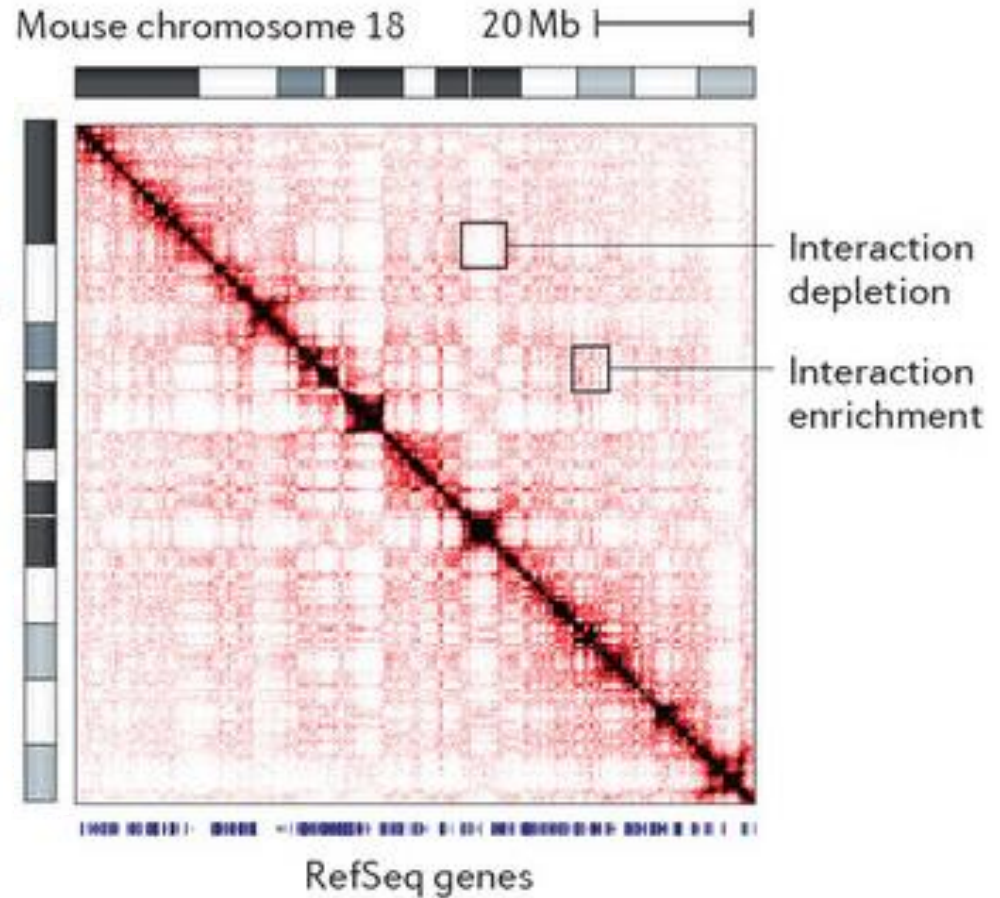
Bait



# Hi-C

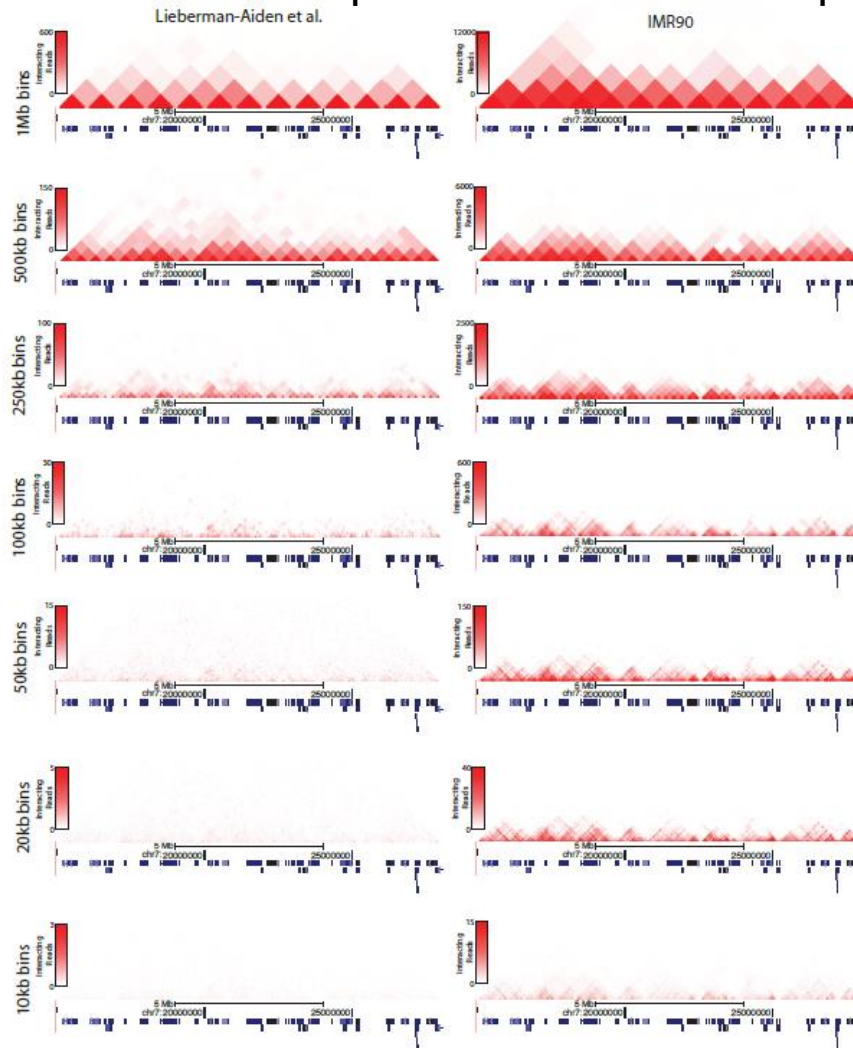


Principle	All against all
Coverage	Genome-wide *
Detection	Paired end HT-sequencing
Resolution	Low *
Limitations	
Examples	All intra- and inter- chromosomal associations



# Resolution is critical

~ 20 million read pairs    ~ 1 billion read pairs

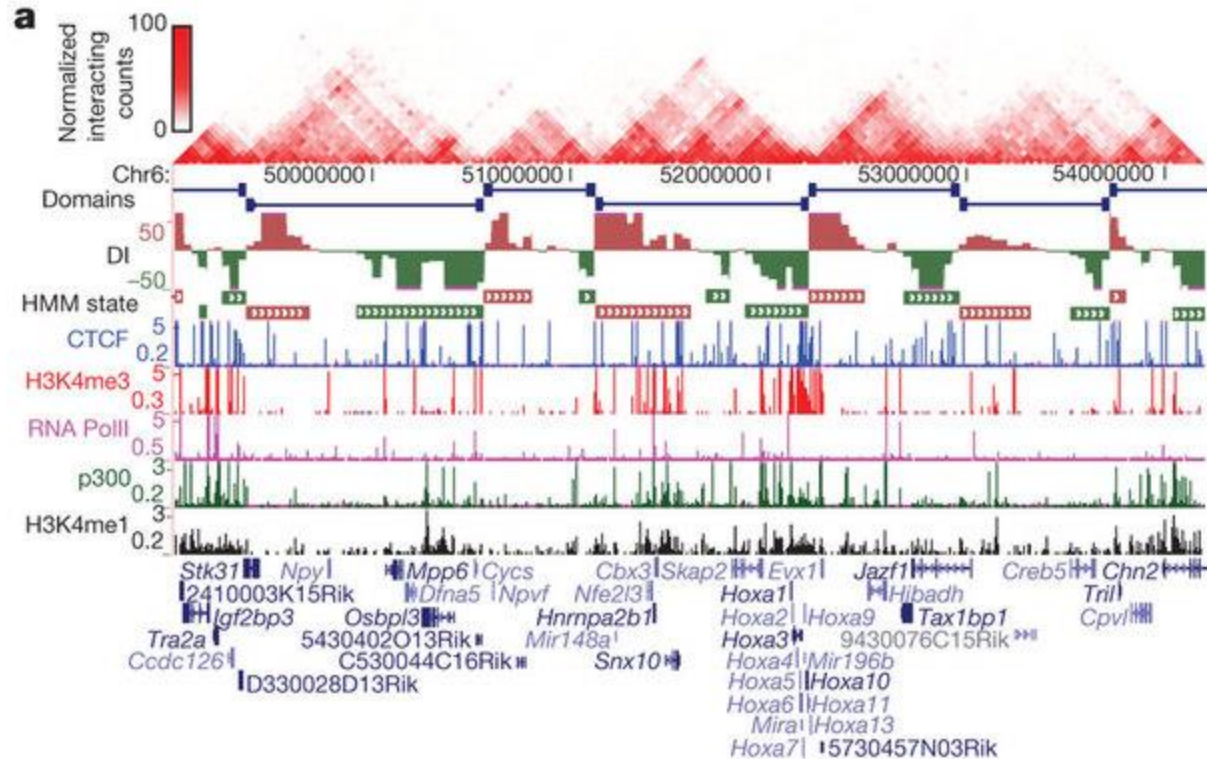


Liebermann-Aiden et al., 2009

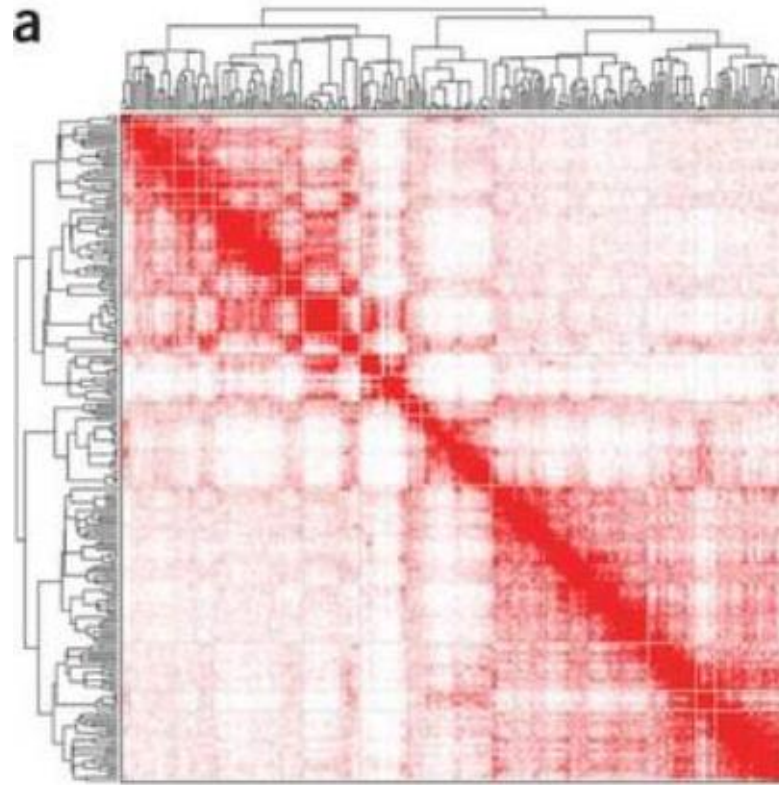
Dixon et al., 2012

# Topological associated domain (TAD)

## Negabase-sized local chromatin interaction domains



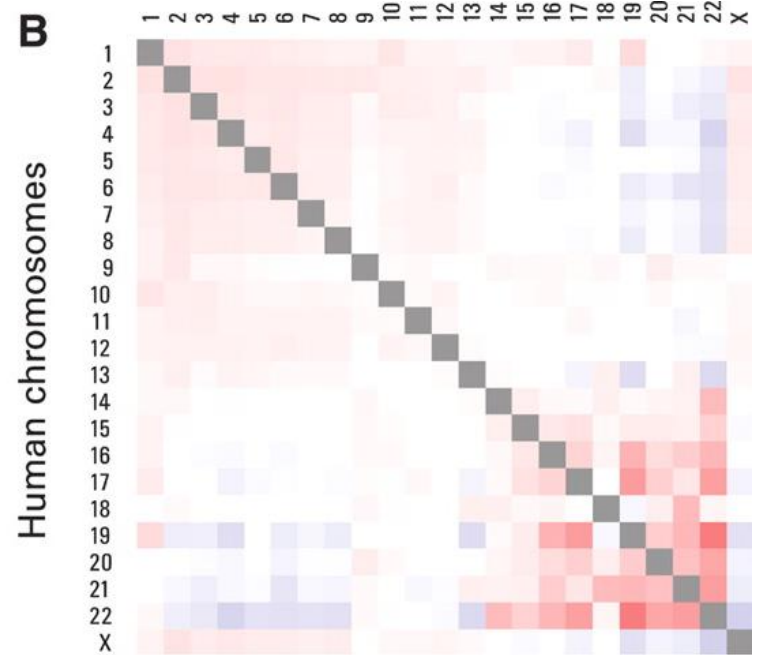
# Domains within domains



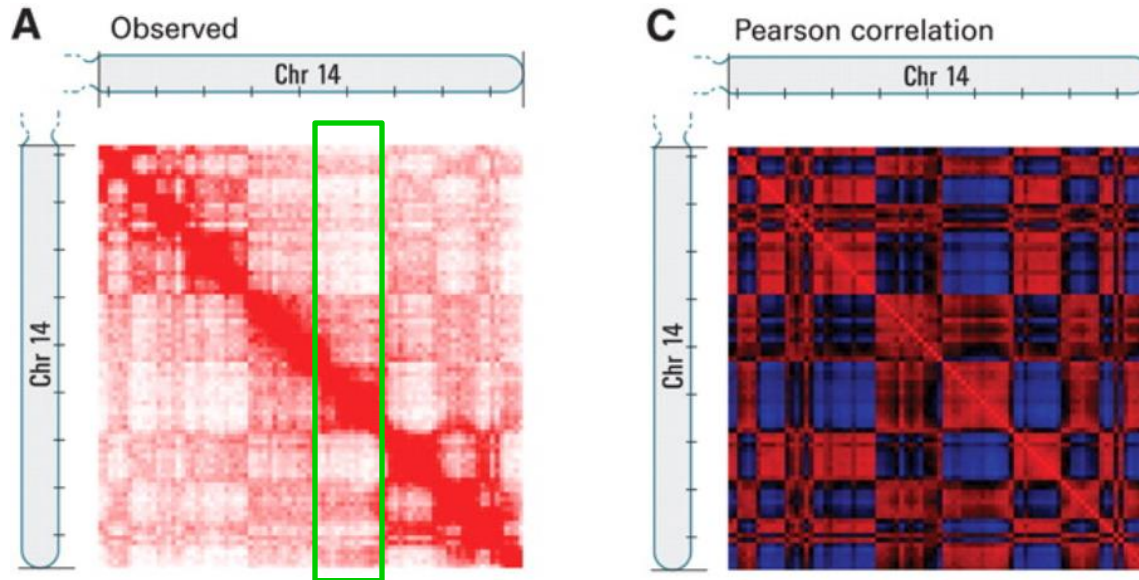
Khalor et al., 2011

# Chromosomes are organized in territories

## Chromosomes are organized in territories



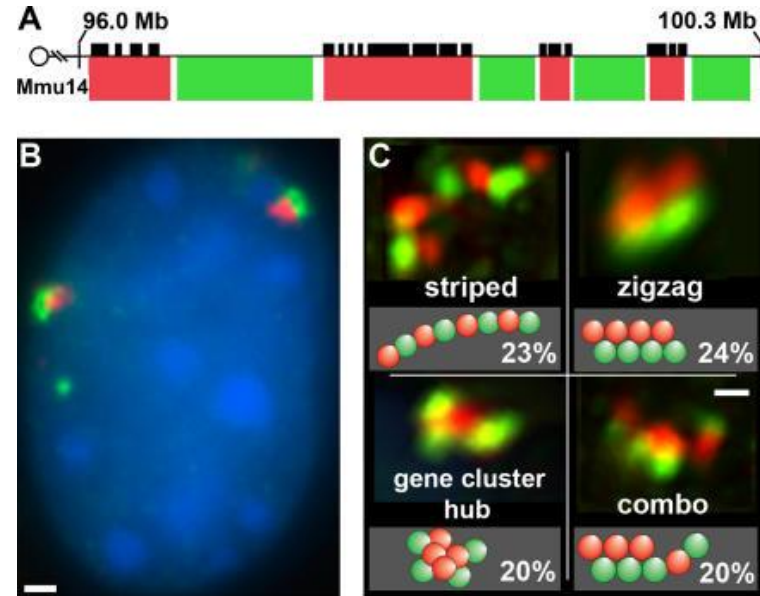
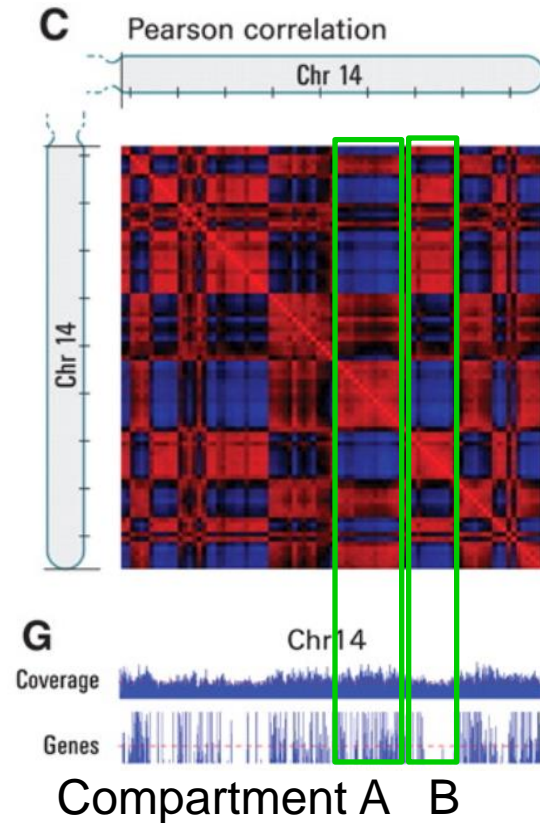
# Preferential contacts within and between chromosomes



**There are two compartments :A and B**

**Are there sub-compartment structures?**

# Genomic associations: Gene density, Gene activity



Compartment A is gene rich

Compartment A

Genes Spearman's  $\rho = 0.431$

Expression Spearman's  $\rho = 0.476$

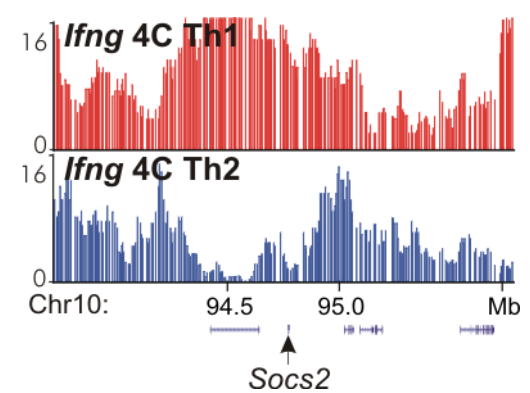
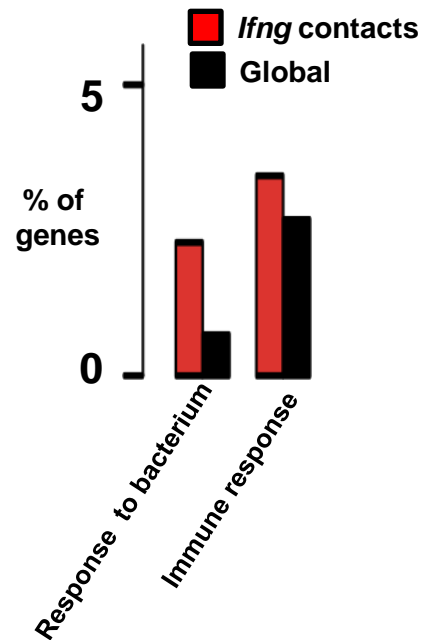
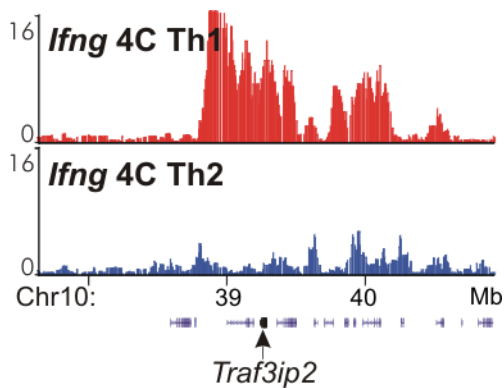
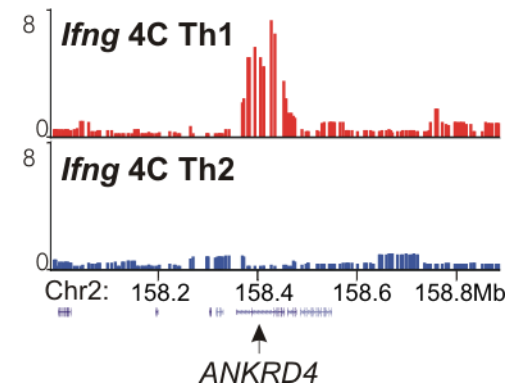
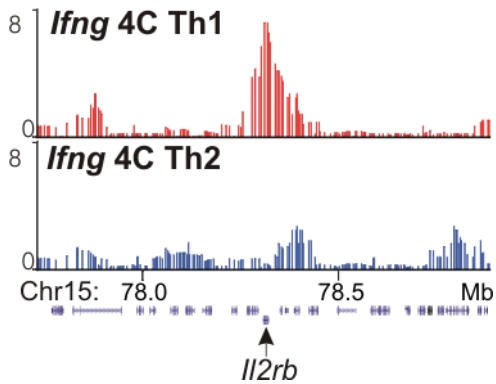
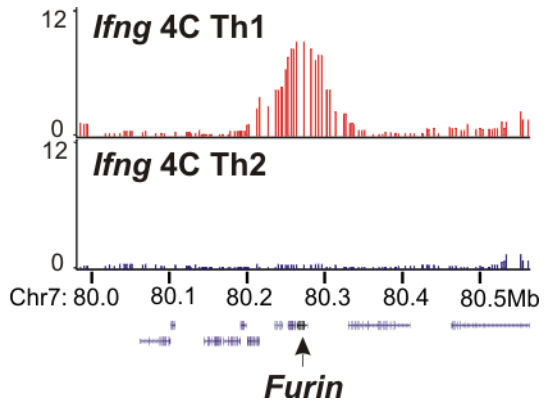
Accessible chromatin, Spearman's  $\rho = 0.651$

H3K36 trimethylation, Spearman's  $\rho = 0.601$  (active)

H3K27 trimethylation, Spearman's  $\rho = 0.282$  (repressive)

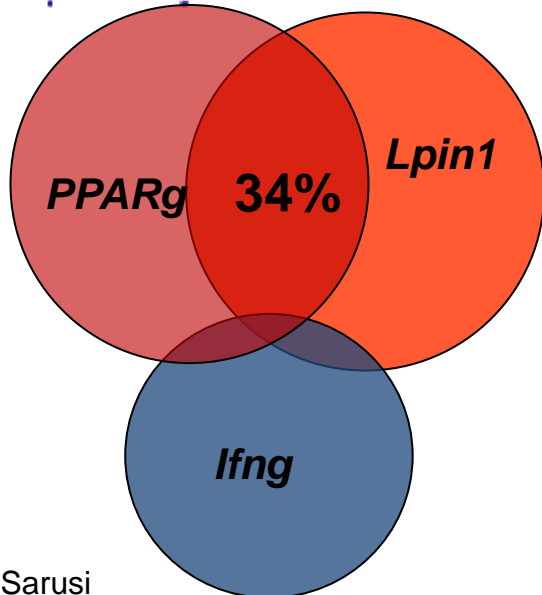
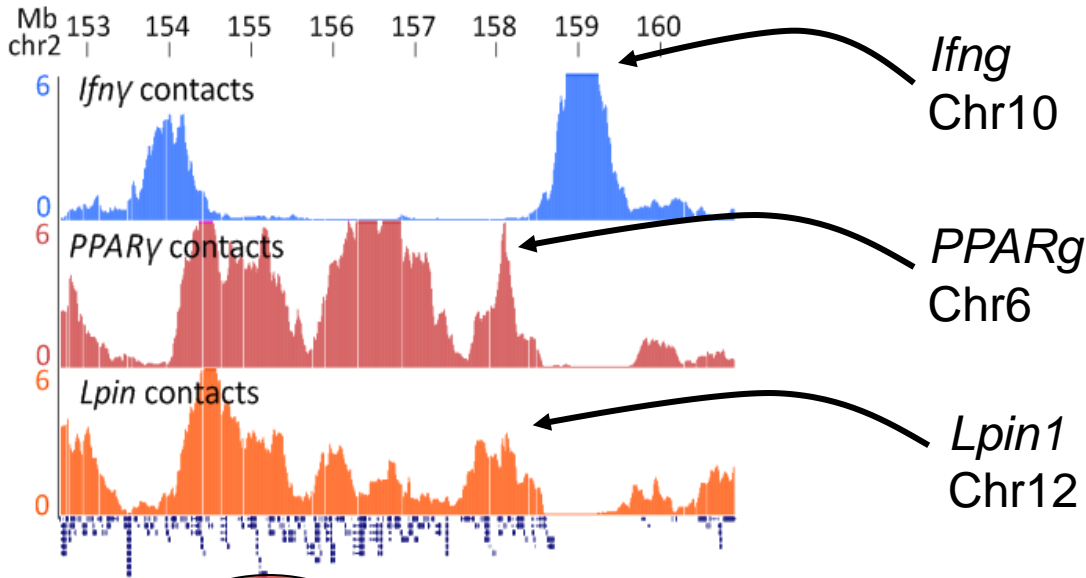
A is more closely associated with open, accessible, actively transcribed chromatin.

# Contacts between TADs are cell-type specific





# Contacts between TADs are cell-type specific



## High enrichment for adipogenic genes

