

Genomic Technologies for studying 3D Genome Organization



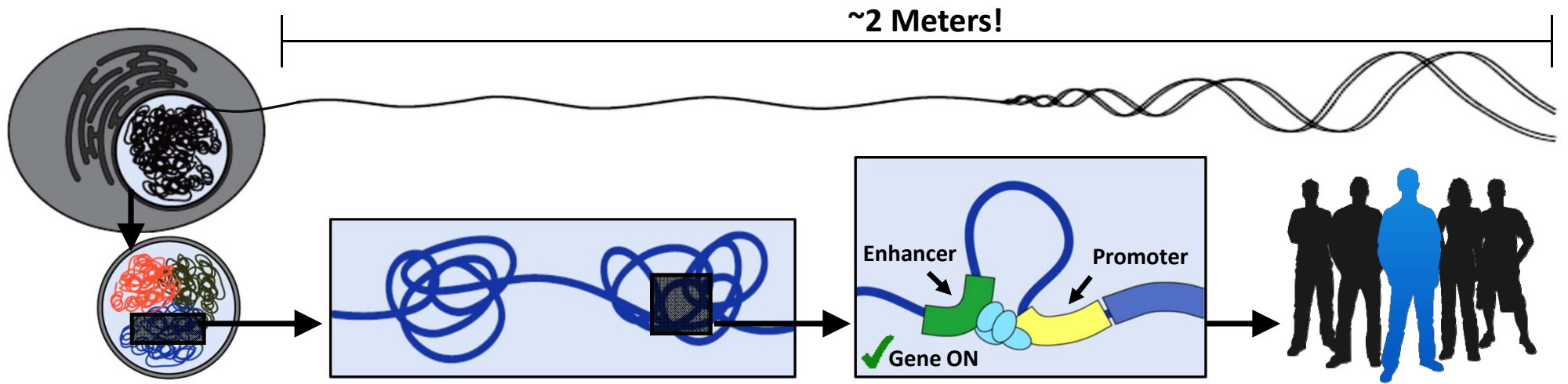
David Gorkin, PhD
Laboratory of Dr. Bing Ren
University of California San Diego
Ludwig Institute for Cancer Research



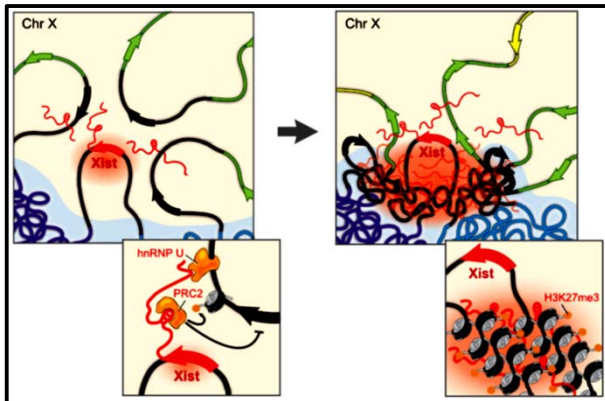
3D Genome Organization and Chromatin Interactions Workshop
ASHG 2017



3D Genome Organization is Integral to Genome Function

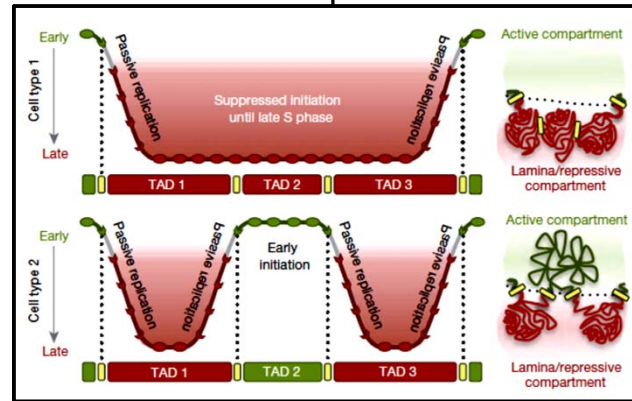


X-chromosome inactivation



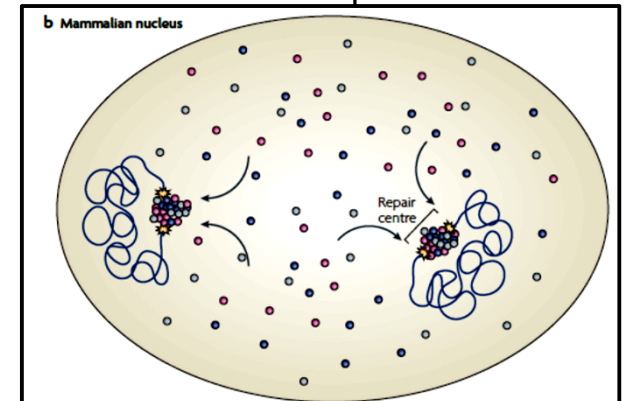
Engreitz et al., Science. 2013

DNA Replication



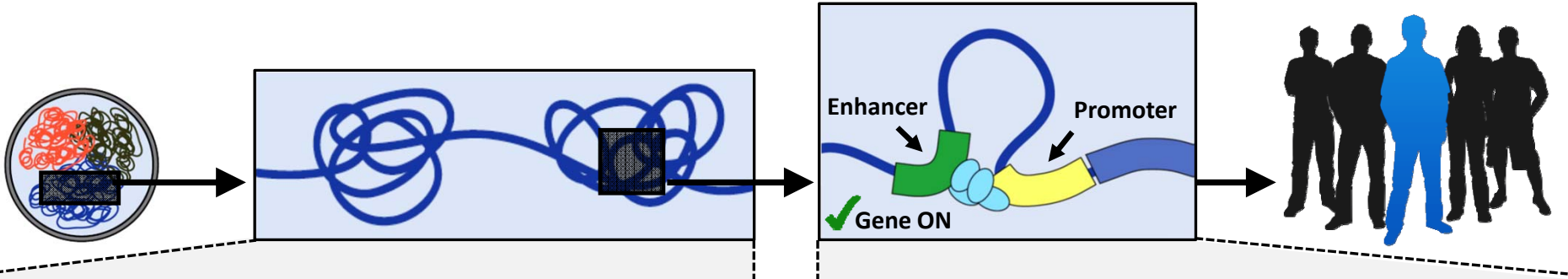
Pope et al., Nature. 2014

DNA repair

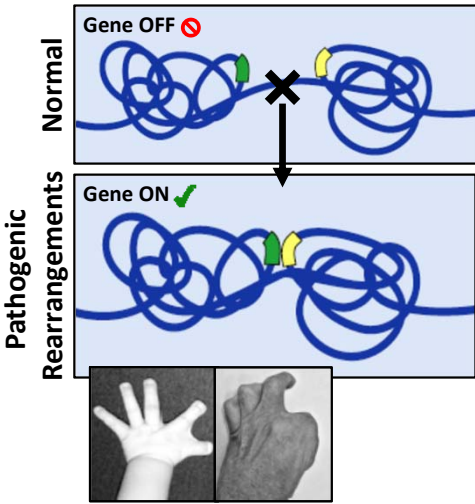


Misteli & Soutoglou, Mol Cell Biol. 2009

3D Genome Organization and *Human Disease*

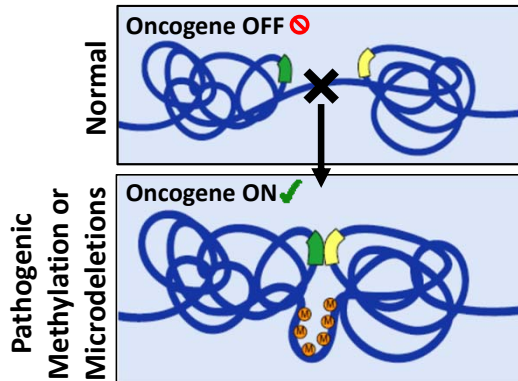


Congenital Malformations

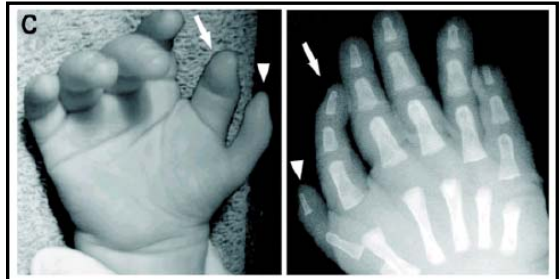
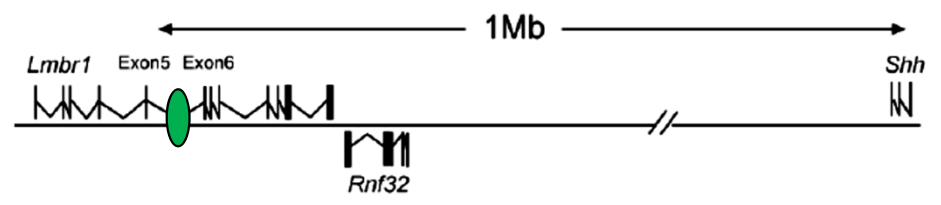


Lupiáñez et al. Cell. 2015;
Franke et al., Nature. 2016

Cancer



Flavahan et al. Nature. 2015;
Hnisz et al. Science. 2016

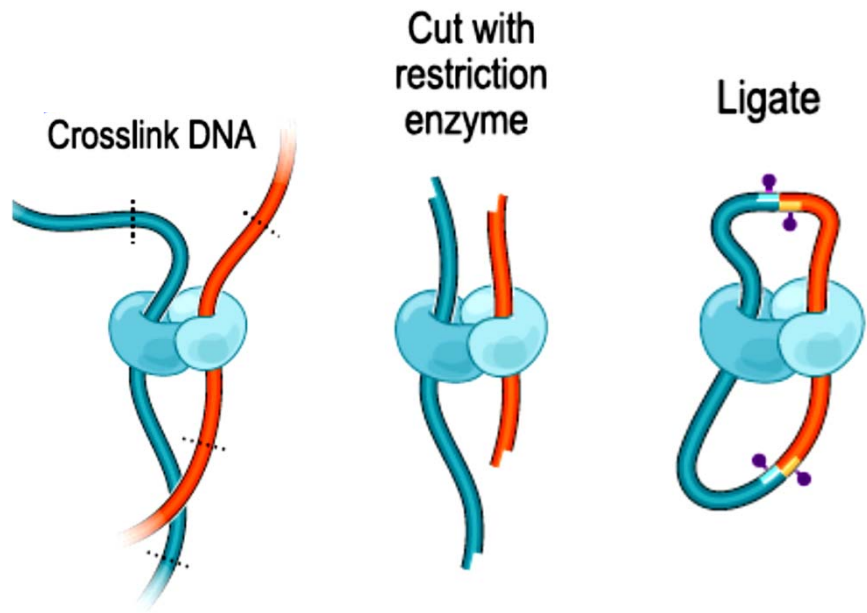
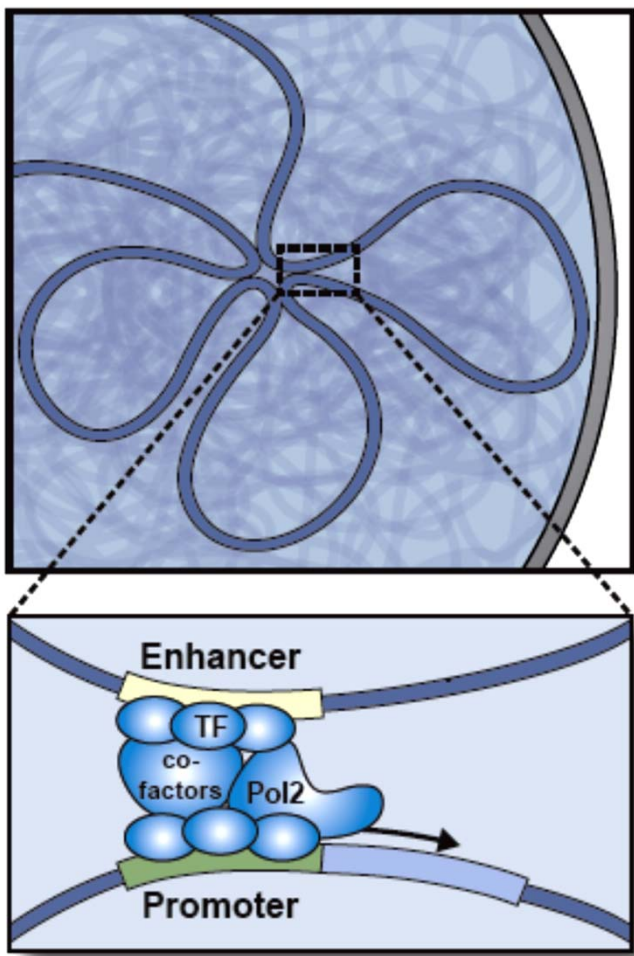


Letteice et al., PNAS. 2002

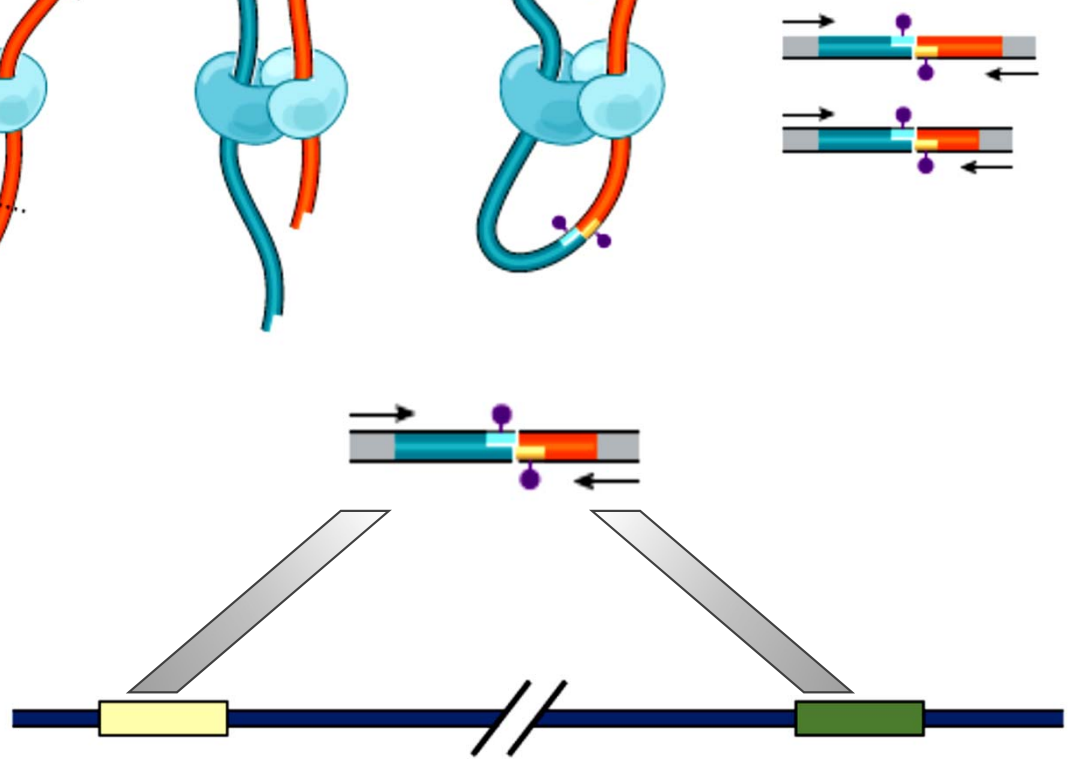


Sagai et al., Development. 2005

Using Proximity Ligation to Assay 3D Genome Organization



Dekker et al., Science.2002
Figures adapted from:
Rao et al., *Cell*. 2014
Gorkin et al. *Cell Stem Cell* 2013



Many variations on the concept of Proximity Ligation

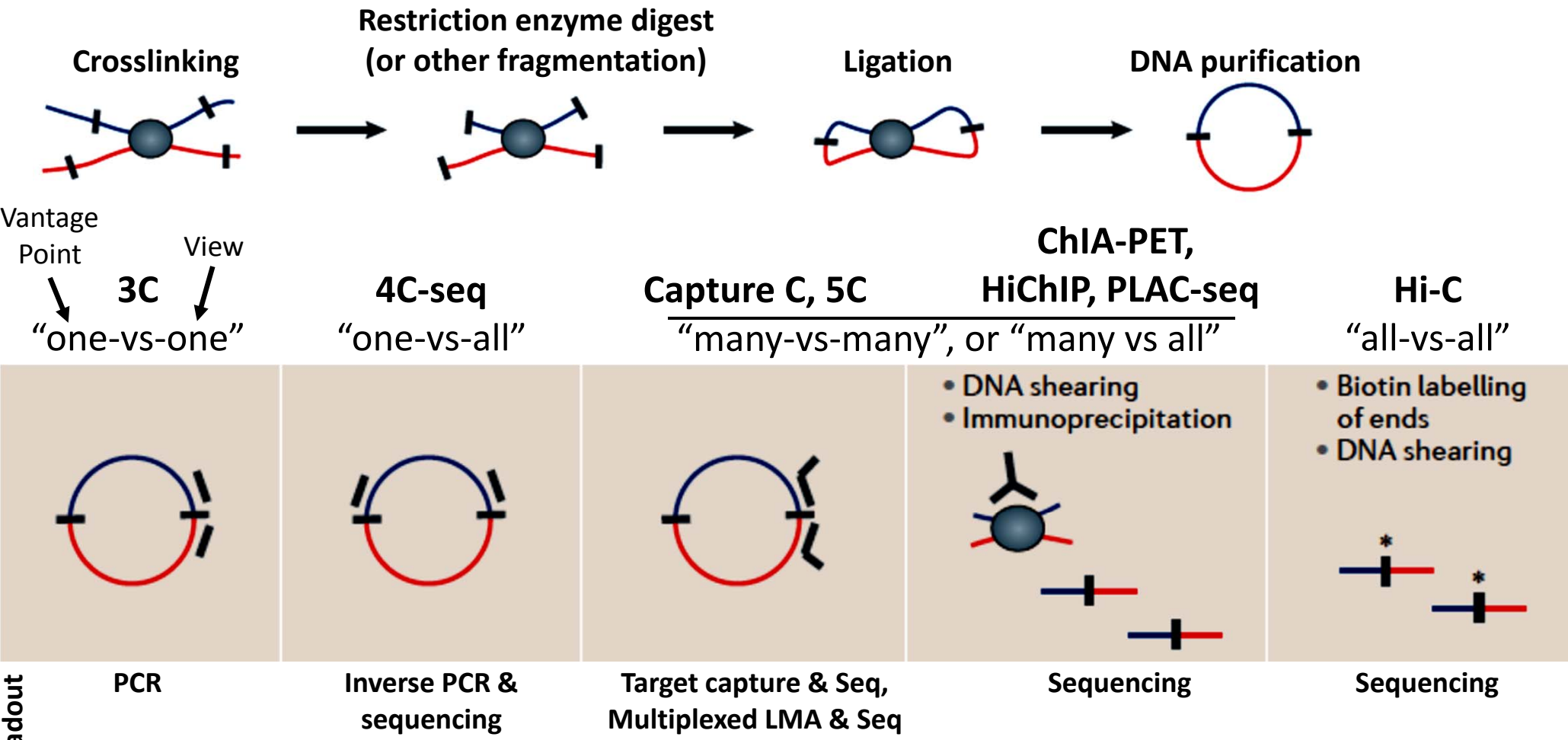
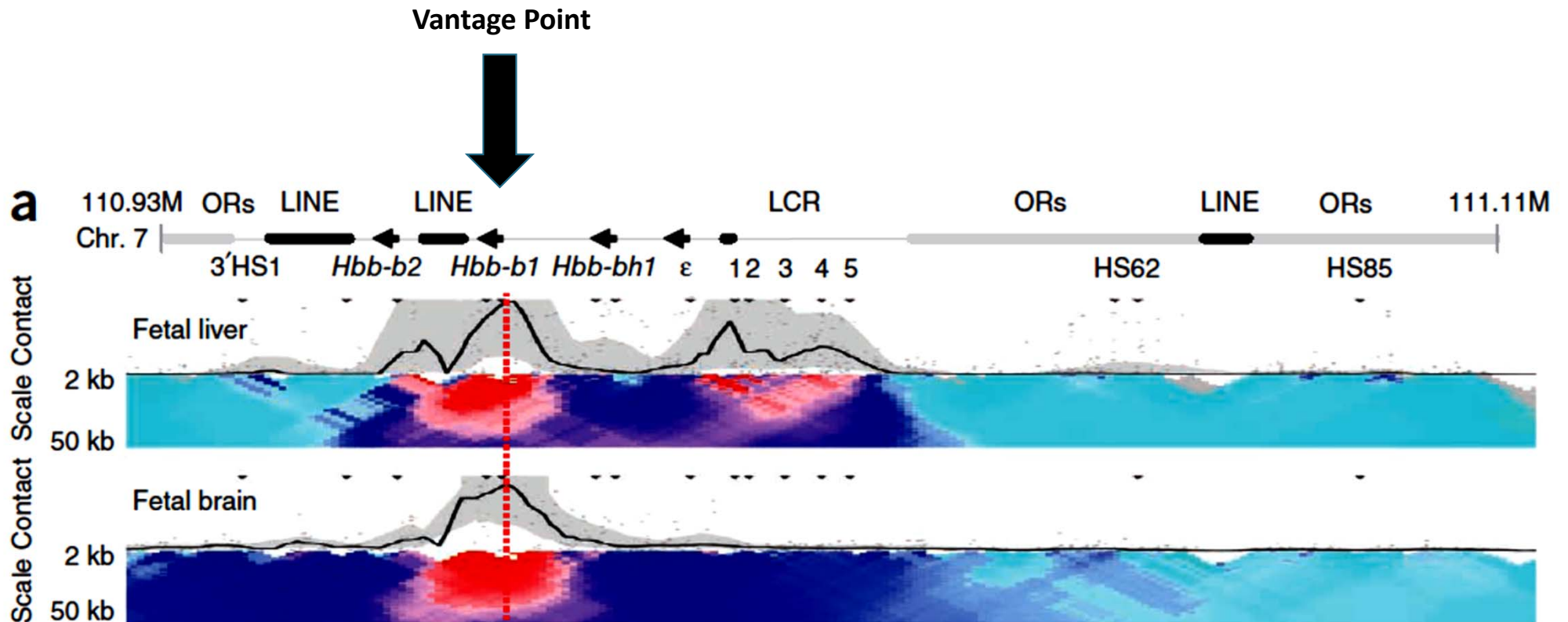


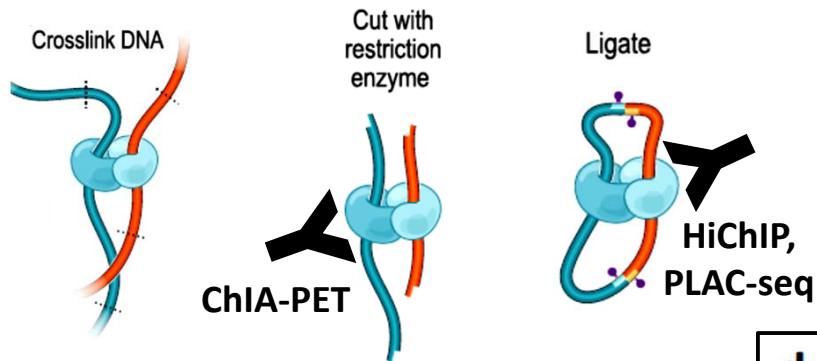
Figure adapted from Dekker, Marti-Ronom, and Mirny. Nat Rev Genet. 2013

4C-seq data: "one vs all"



ChIA-PET, HiChIP, PLAC-seq: “many-vs-many”, or “many-vs-all”

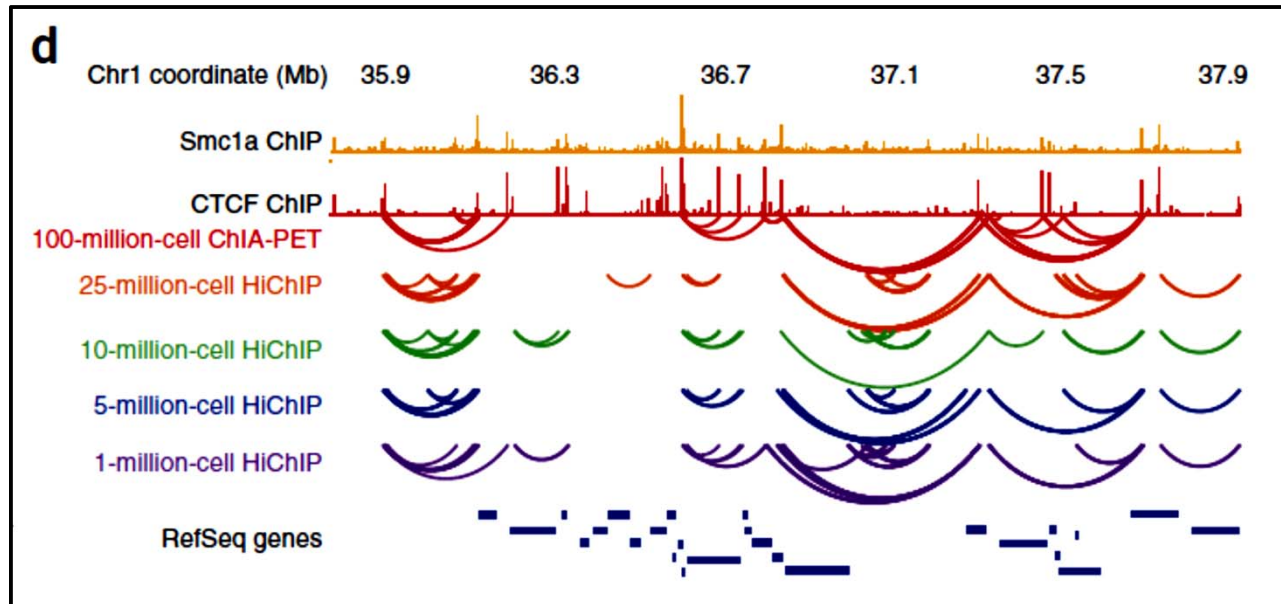
Proximity Ligation + IP for protein of interest



HiChIP: Mumbach et al. Nat Methods. 2016

PLAC-seq: Fang, Yu, et al. Under review.

(bioRxiv 074294; doi: <http://dx.doi.org/10.1101/074294>)



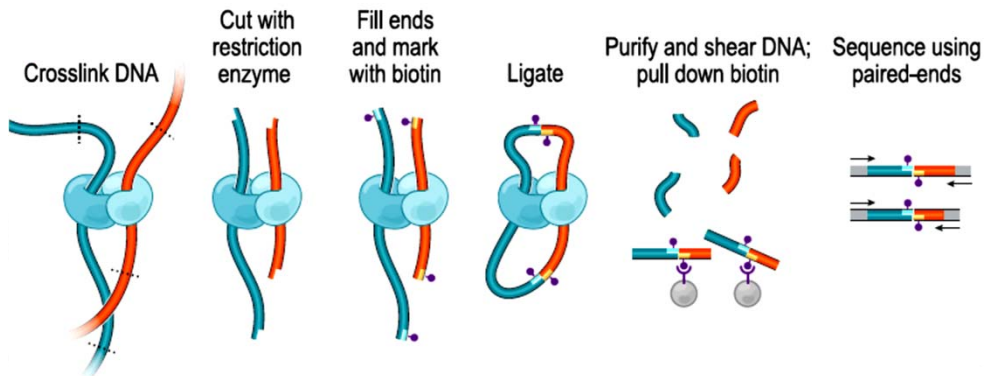
Mumbach et al. Nat Methods. 2016

Hi-C data: “all-vs-all”

Lieberman-Aiden, et al. Science. 2009, Rao et al. Cell. 2014

Hi-C

High throughput Chromatin Conformation Capture



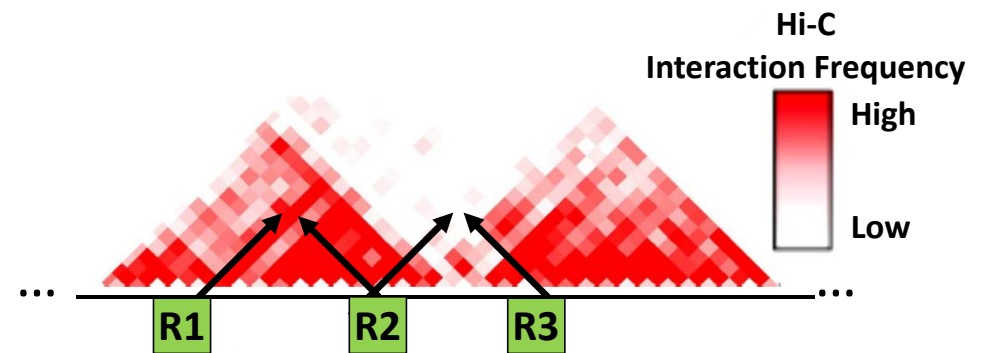
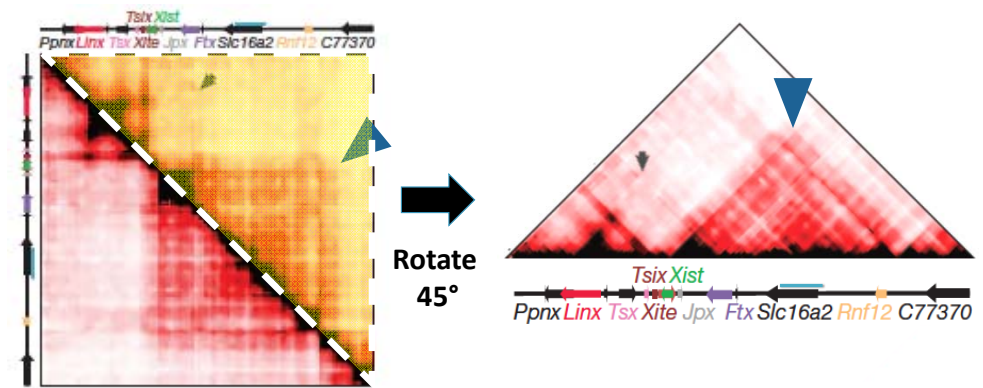
Restriction enzyme

- 6-cutter (~5Kb)
- 4-cutter (~250bp)

Sequencing depth

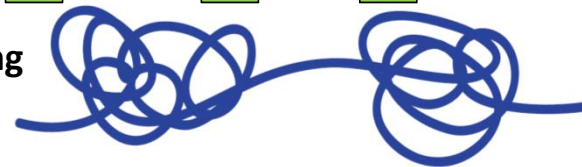
- ~300-500M pairs standard (40Kb)
- ~1B+ pairs “high-res” 10Kb+

Factors that determine resolution

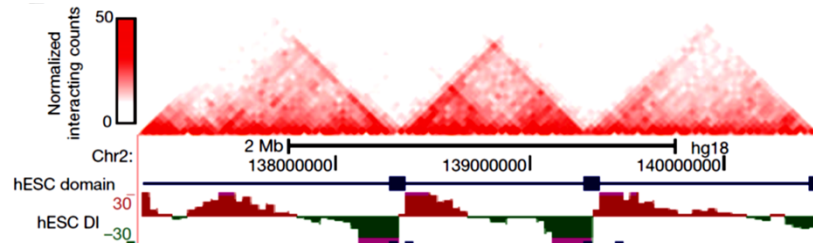
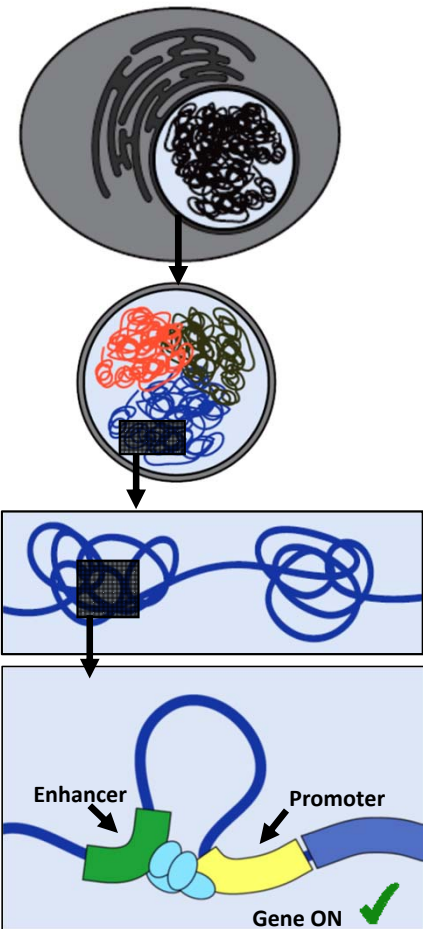


Topologically Associating Domains (TADs)

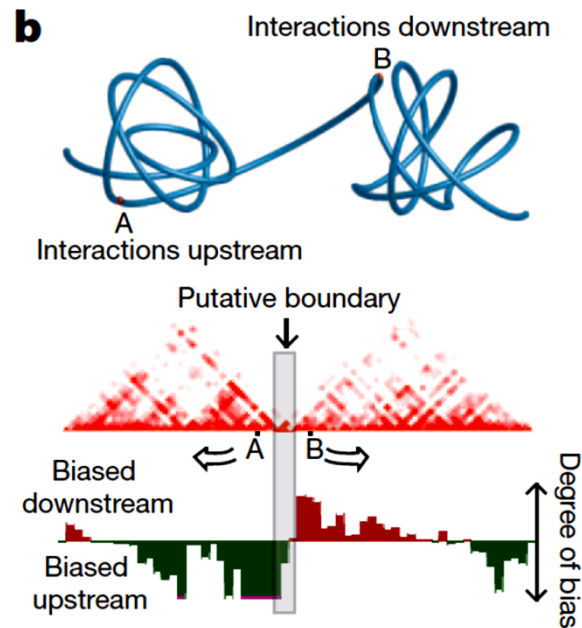
Nora et al. Nature. 2012
Dixon et al. Nature. 2012



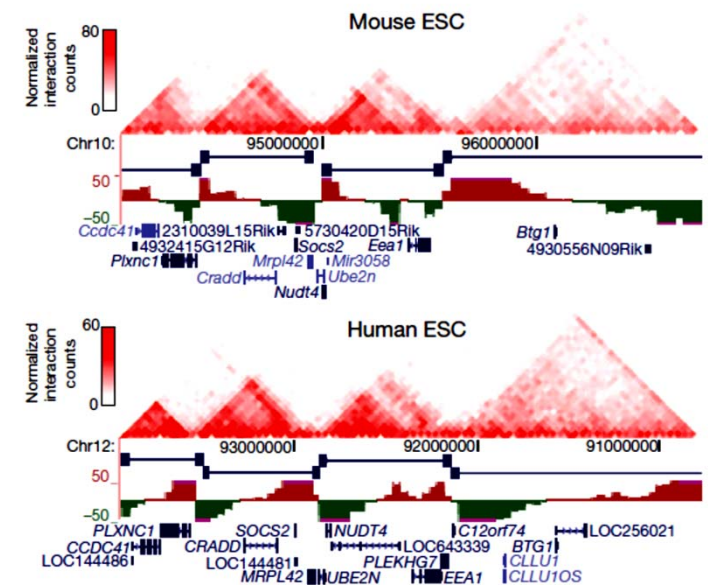
A bit more about *Topologically Associating Domains (TADs)*



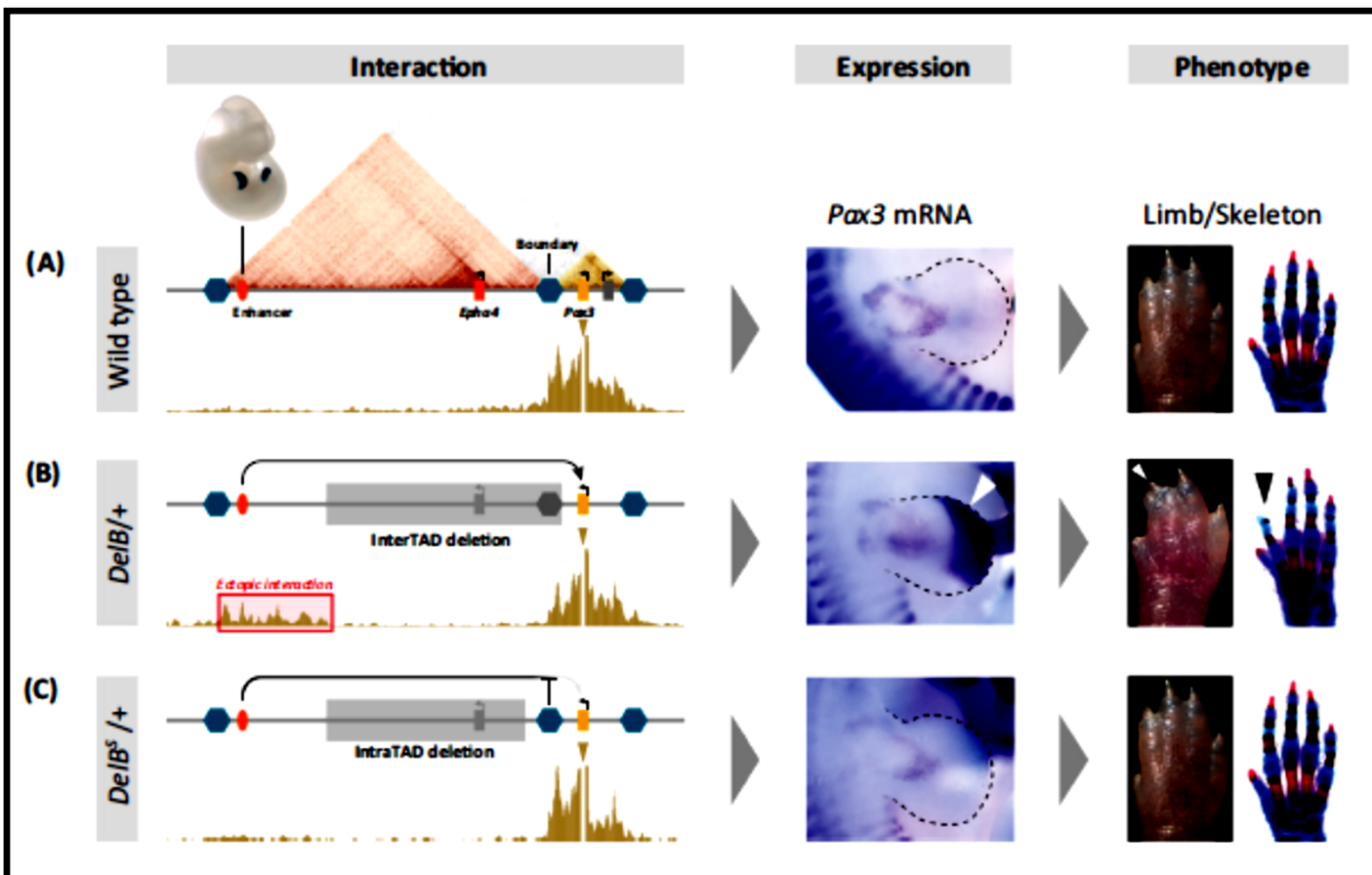
- Genome is organized into ~2000 TADs (1-2Mb)
- Highly consistent between cell types
- CTCF & other mechanisms maintain boundaries
- Highly conserved from mouse to human



Dixon et al. Nature. 2012

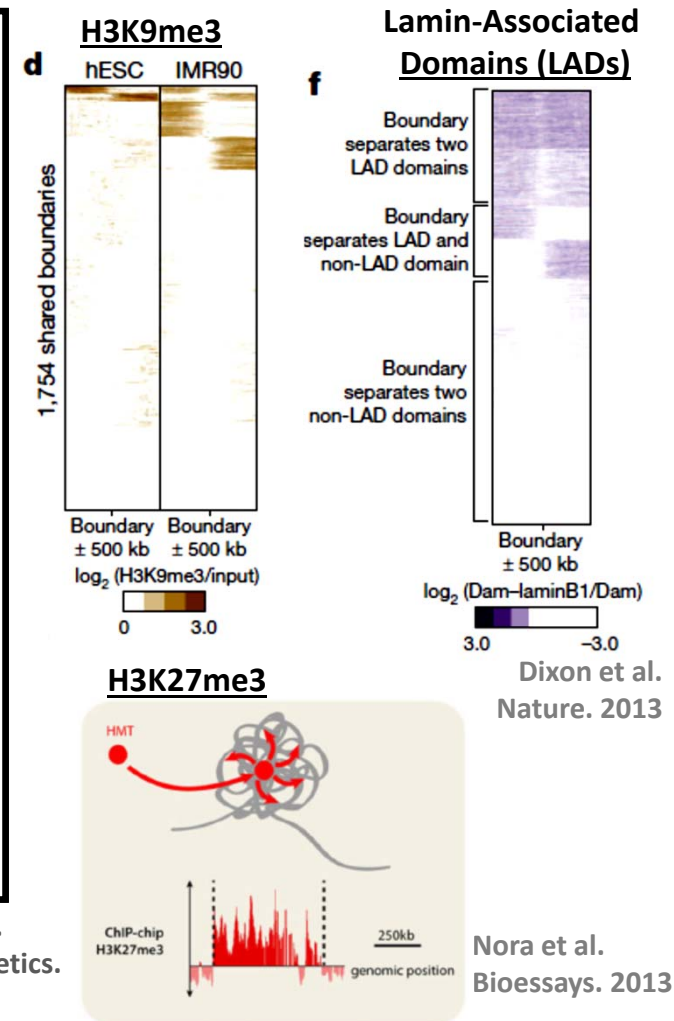


Topologically Associating Domains (TADs) as *regulatory units*



- TADs constrain Enhancer-Promoter interactions
- TADs divide the genome functional units that are often co-regulated

Lupiáñez et al.
 Trends in Genetics.
 2016



Summary

- **3D Genome is Integral to genome function & gene regulation.**
- **Proximity ligation has given rise a series of genomic technologies to assay 3D genome organization.**
 - Differ in scope, scale, and resolution.
- **Key discovery: The interphase genome is organized into TADs**
 - Important to consider TADs when thinking about the consequences of structural and non-coding mutations (or variants).

Acknowledgements

ENCODE: San Diego Data Production Group

Dr. Bing Ren

Dr. Joe Ecker

Dr. Len Penacchio

Dr. Axel Visel

Dr. Wei Wang

4DN: San Diego 4D Nucleome Center

Dr. Bing Ren

Dr. Cornelis Murre

Dr. Ana Pombo

Dr. Olga Dudko

Dr. Mario Nicodemi

Dr. Ming Hu

Ren Group

Dr. Bing Ren

Yunjiang Qiu

Dr. Anthony Schmitt

Dr. Inkyung Jung

Dr. Siddarth Selvaraj

Dr. Jesse Dixon

Dr. Bin Li

Ah Young Lee

Zhen Ye

Samantha Kuan

1KG Collaborators

Dr. Charles Lee

Dr. Jonathan Sebat

Dr. Amina Noor

