Breaking the Hi-C Resolution Barrier

Micro-C – enabling chromatin conformation capture at the nucleosome level

Cory Padilla, Ph.D.
Head of Scientific Affairs

- **25% Discount on Kits**
  - Micro-C Kit
  - HiChIP Mnase Kit
  - Omni-C Kit (Restriction-enzyme free Hi-C)
- **Epigenetic Service** (Lib Prep and optional sequencing)
  - Micro-C libraries
  - HiChIP with CTCF, H3K4me3, or H3K27ac

Kai Kleiber, PhD
Kai.kleiber@dovetail-genomics.com
UK: +44 7737 127 172
GER: +49 157 3077 3367

If you are interested in ChIP with HiChIP, Capture-C or different Antibodies, please ask

Outline

- Chromatin Conformation
  - Why conformation matters
  - Introduction to chromatin conformation capture
- Limitations of Current Hi-C approaches
- Micro-C Breaks the Resolution Barrier
- Further Leveraging Micro-C in Epigenetics

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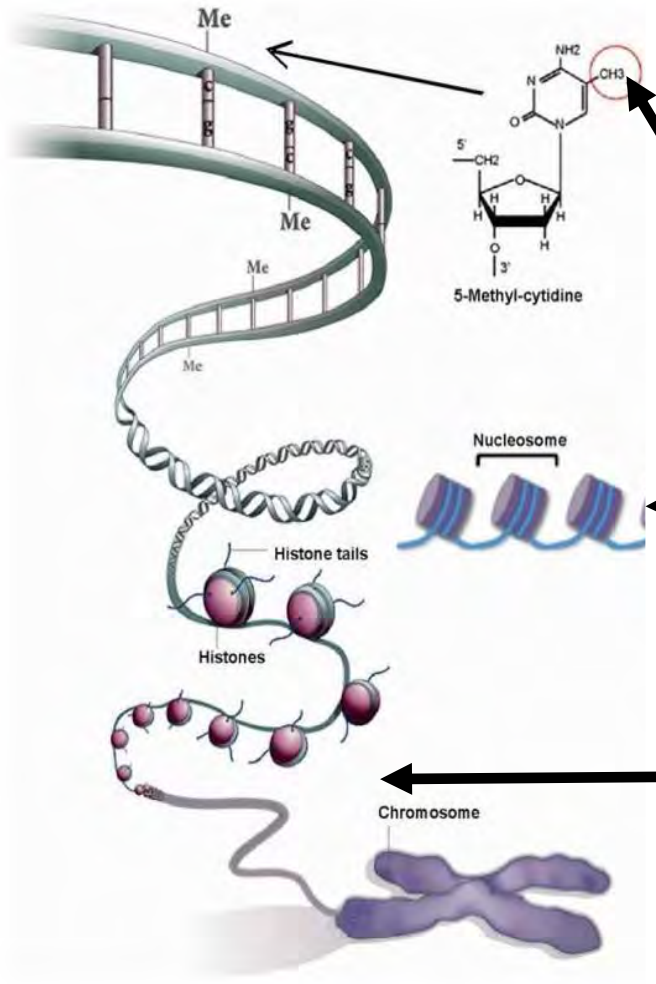
Chromatin Conformation

Today's Epigenetics Is Linear

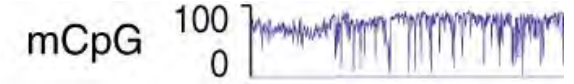
Heavily rooted in primary order features

- Methylation
- Histone Modifications
- Chromatin Accessibility

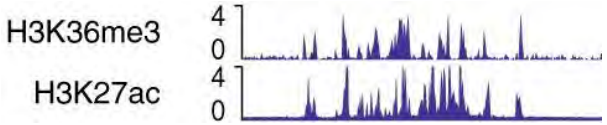
Current methods result in linear views of the genome



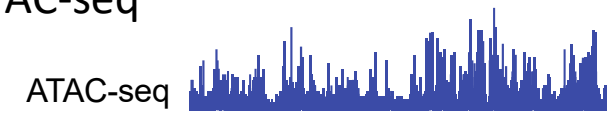
Bisulfite Sequencing



ChIP-seq



ATAC-seq



Genomes Are Not Organized In A Straight Line

Three ways genome conformation influences gene expression:

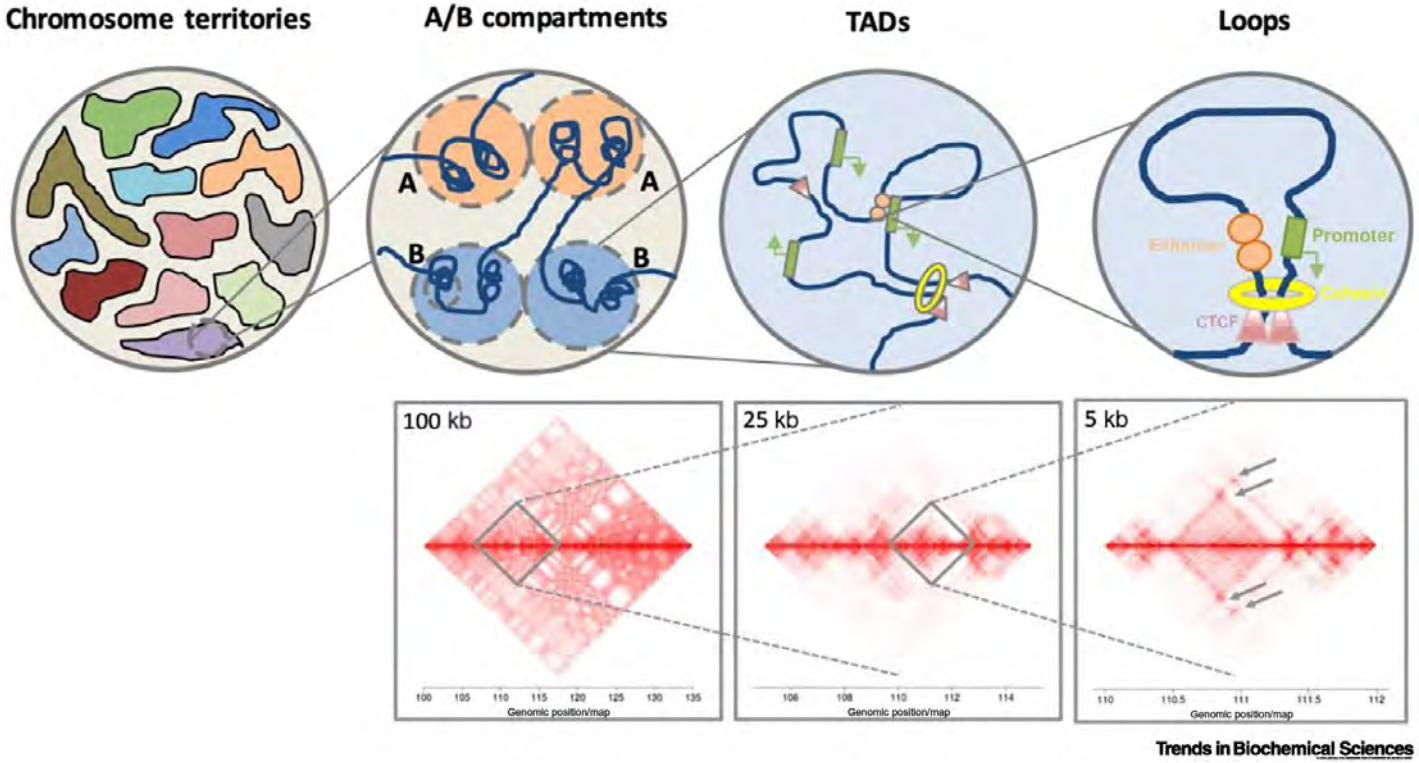
1. Chromatin found in:
   - Active compartments (A)
   - Heterochromatic inactive compartments (B)

2. Gene clusters of linked function in TADs

3. Mediates transcription factor interaction with target genes

Conformation provides a view into the transcriptional landscape of chromatin

Hierarchical genome conformation features



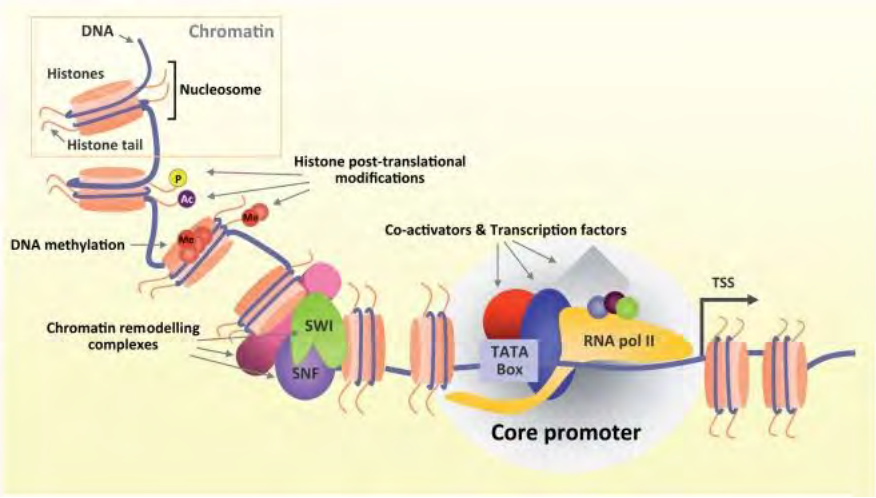
Trends in Biochemical Sciences

Write et al., 2019

How does conformation help us understand the transcriptional landscape?

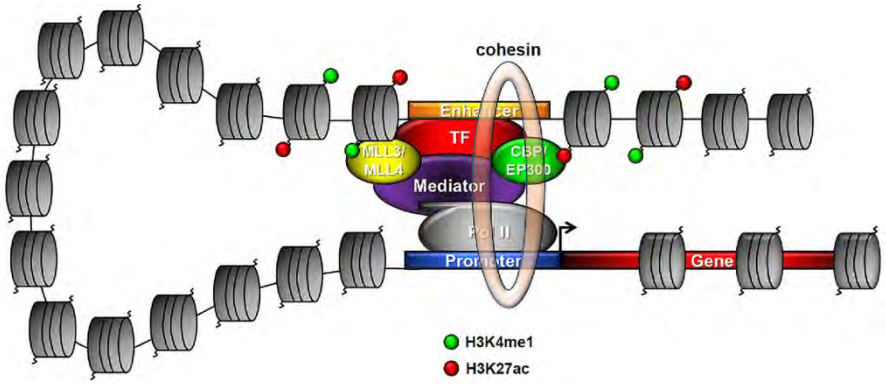
- It enables us to assess what part of the chromatin is physically close in 3D space
- When used in combination with known locations of genomic and epigenomic features (promotors, transcription factors, enhancers, protein binding) it enables us to interpret the importance of these chromatin folding features in light of transcriptional activity

Linear View of Gene Transcription

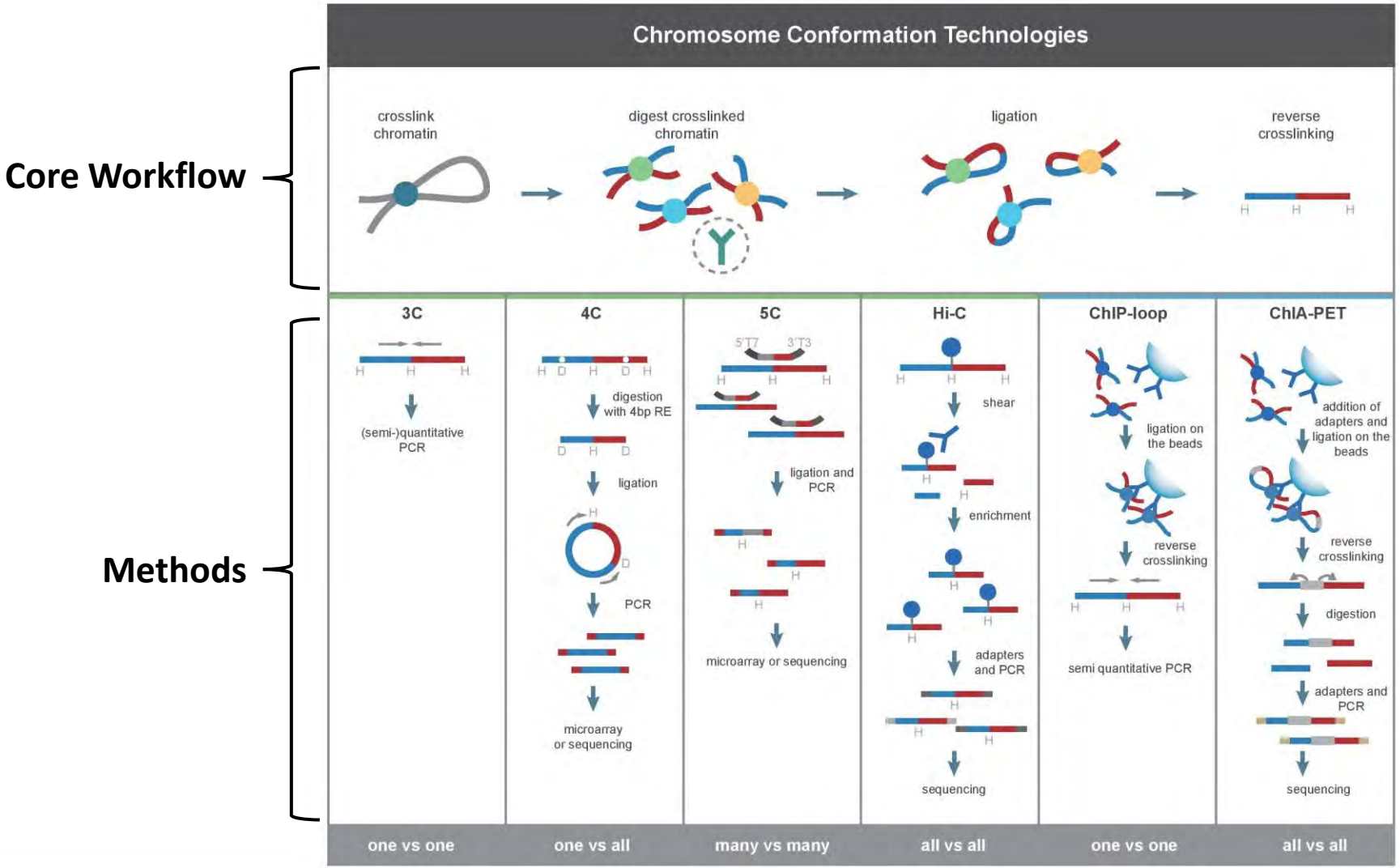


Proximity-ligation

3D View of Gene Transcription



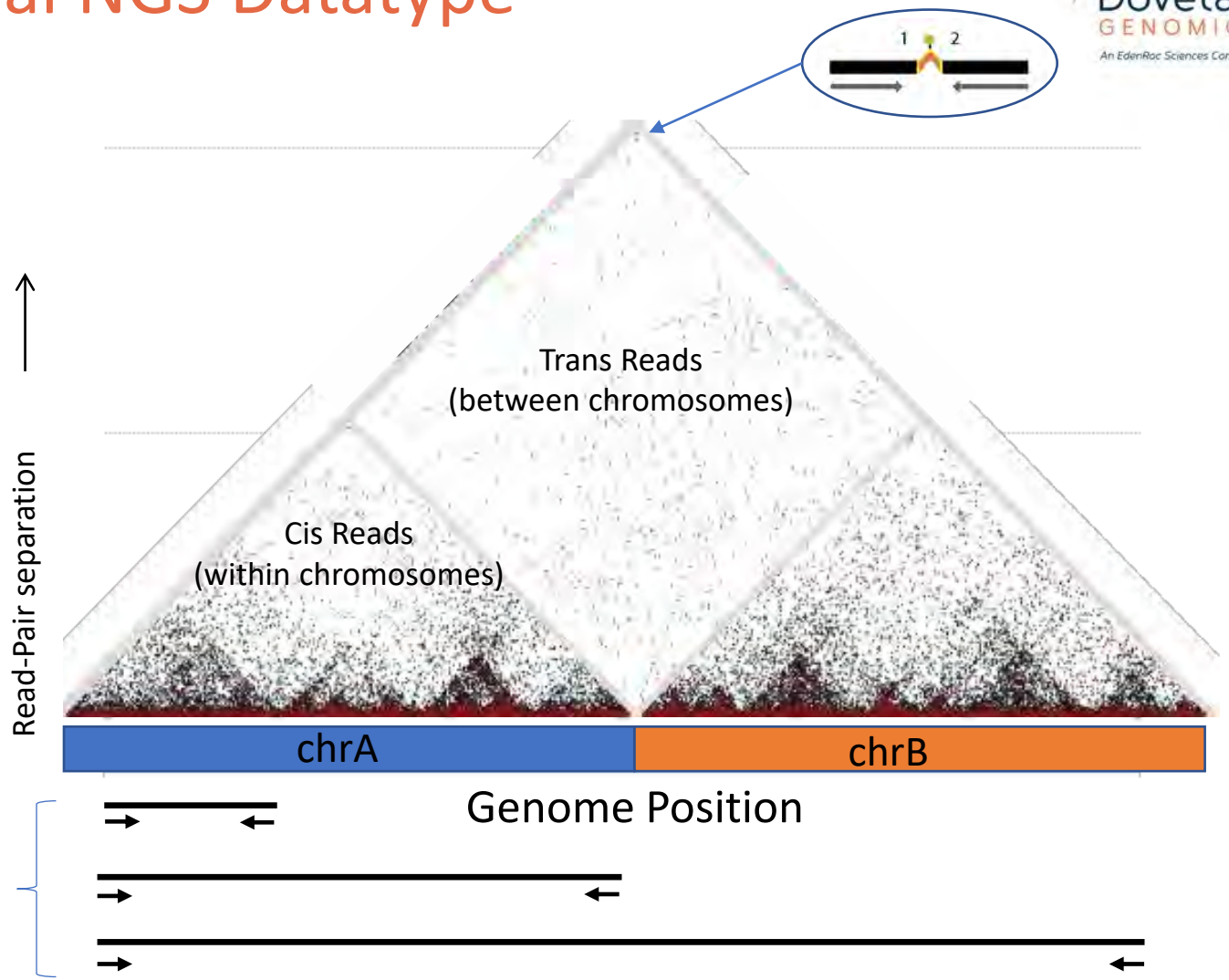
The World Of Chromatin Conformation Capture



Li *et al.*, 2014

Powerful Multi-Dimensional NGS Datatype

Read-pairs span distances from single base pairs to megabases



Read-pairs of varying lengths enable you to create a scaffold of the genome

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Limitations of Current Hi-C Approaches

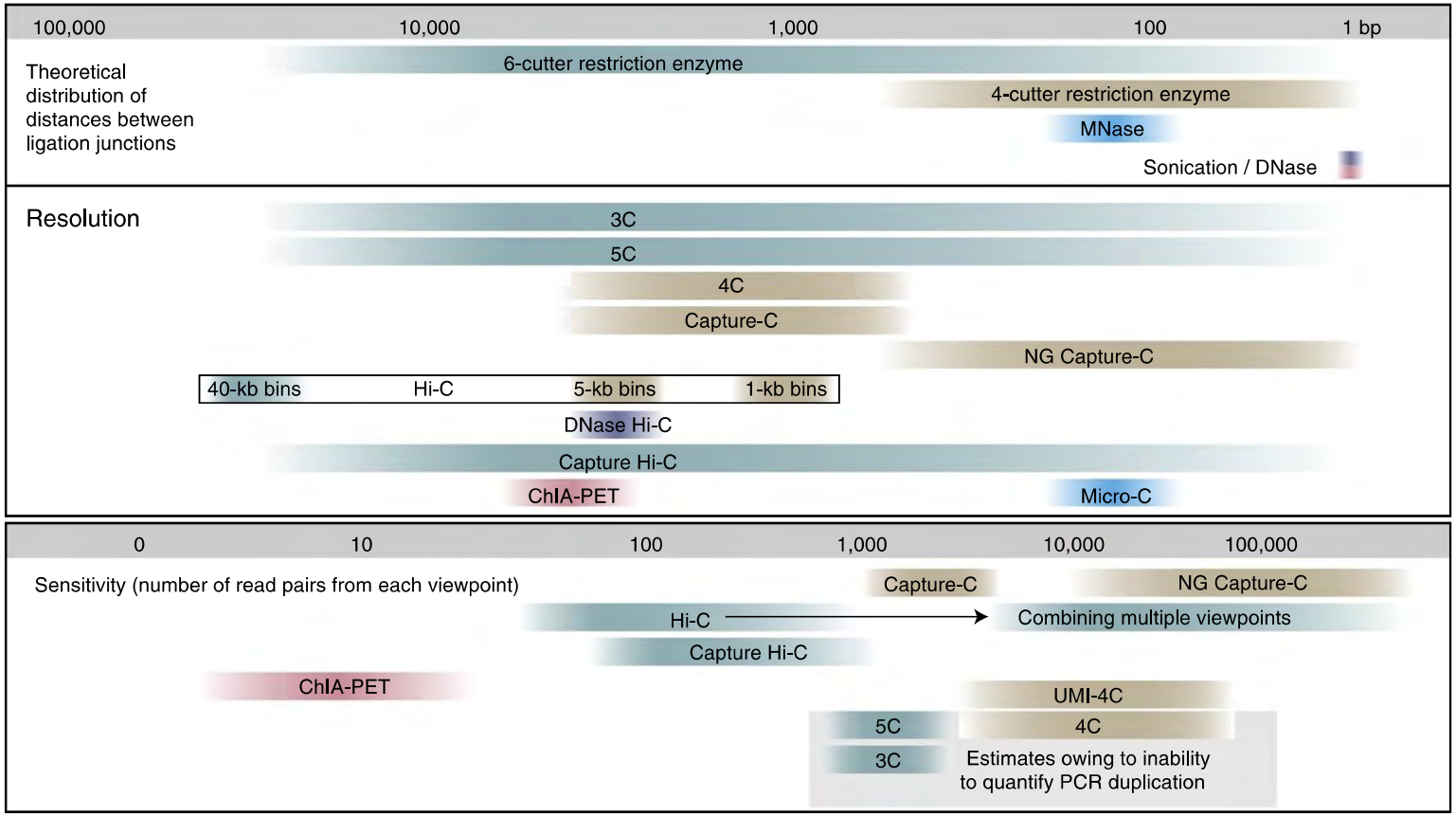
The Fundamental Limitation of RE-based Hi-C Approaches is Resolution

Resolution – how far can you zoom in on conformation features?

Functional resolution – Fragment Size

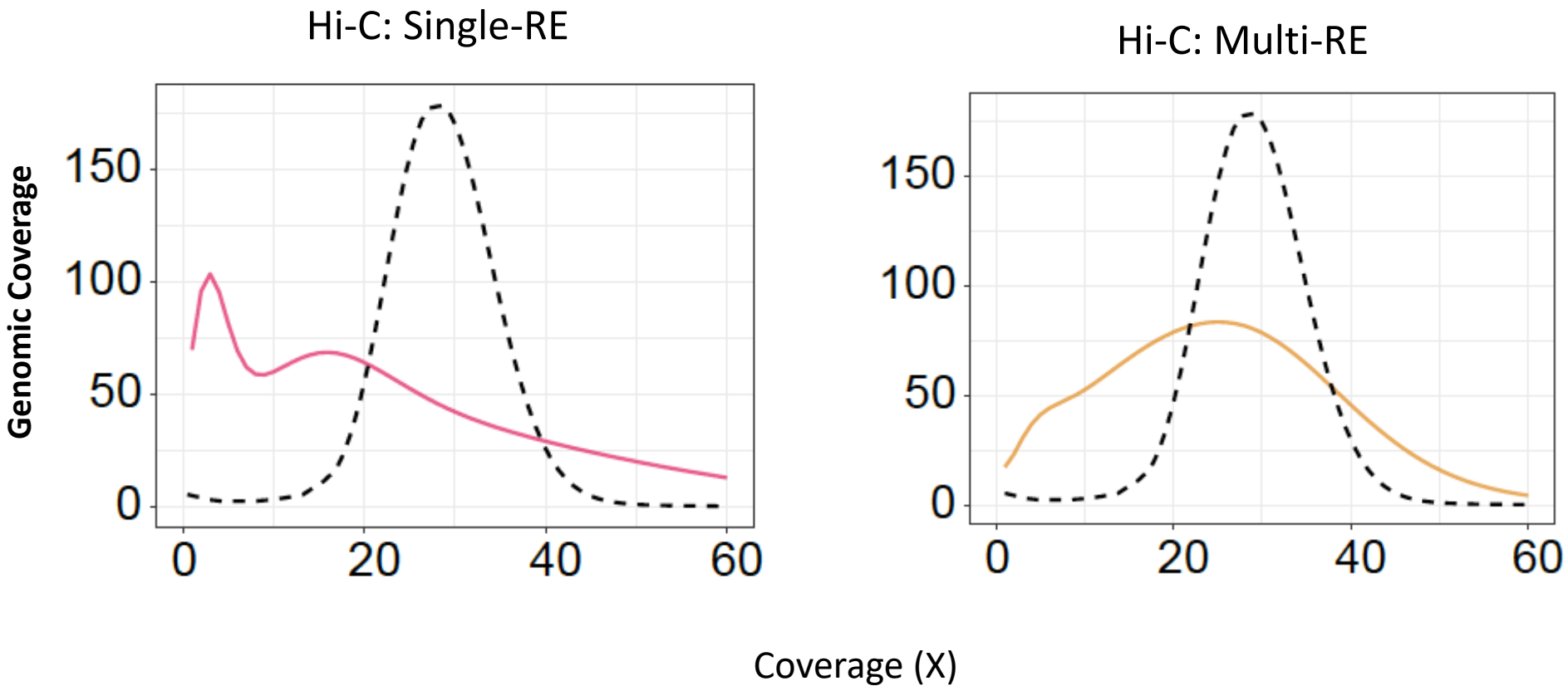
Contact Matrix resolution – Read-support per fragment

The upper limit of RE-based Hi-C is 1 kb in conformation studies



Davies et al., 2017

Restriction Enzyme Approaches Limit Genomic Coverage and Reduces Sequencing Efficiency



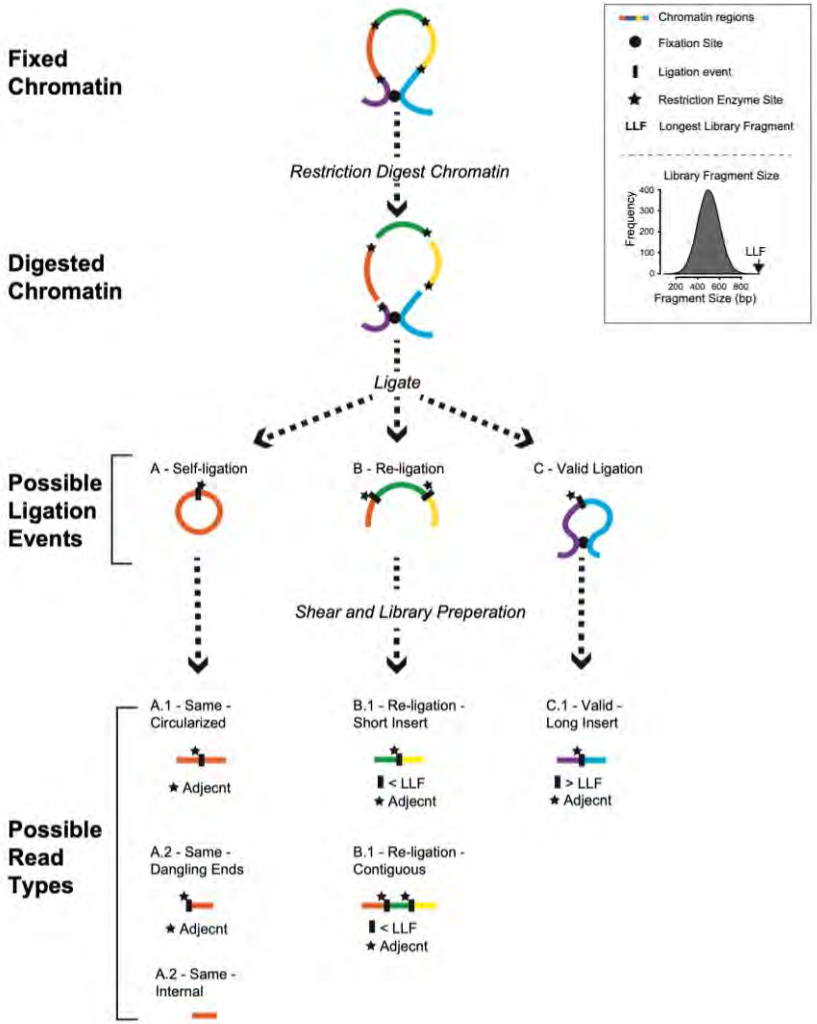
Larger Fragment Size in RE-based Hi-C Limit the Amount of Useable Reads

During Proximity three main ligation events can occur

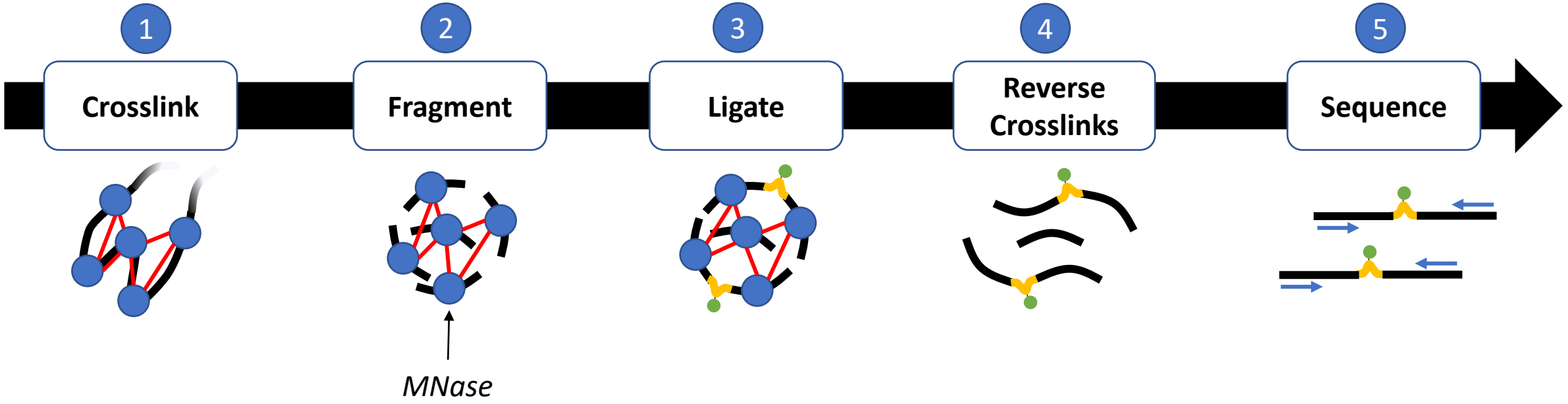
- Self-circularized
- Re-ligation
- Valid Ligation

Long fragments generated by RE's require sheering for library conversion

Leads to the incorporation of more non-informative reads in the data



Dovetail™ Micro-C Libraries Set New Hi-C Standard

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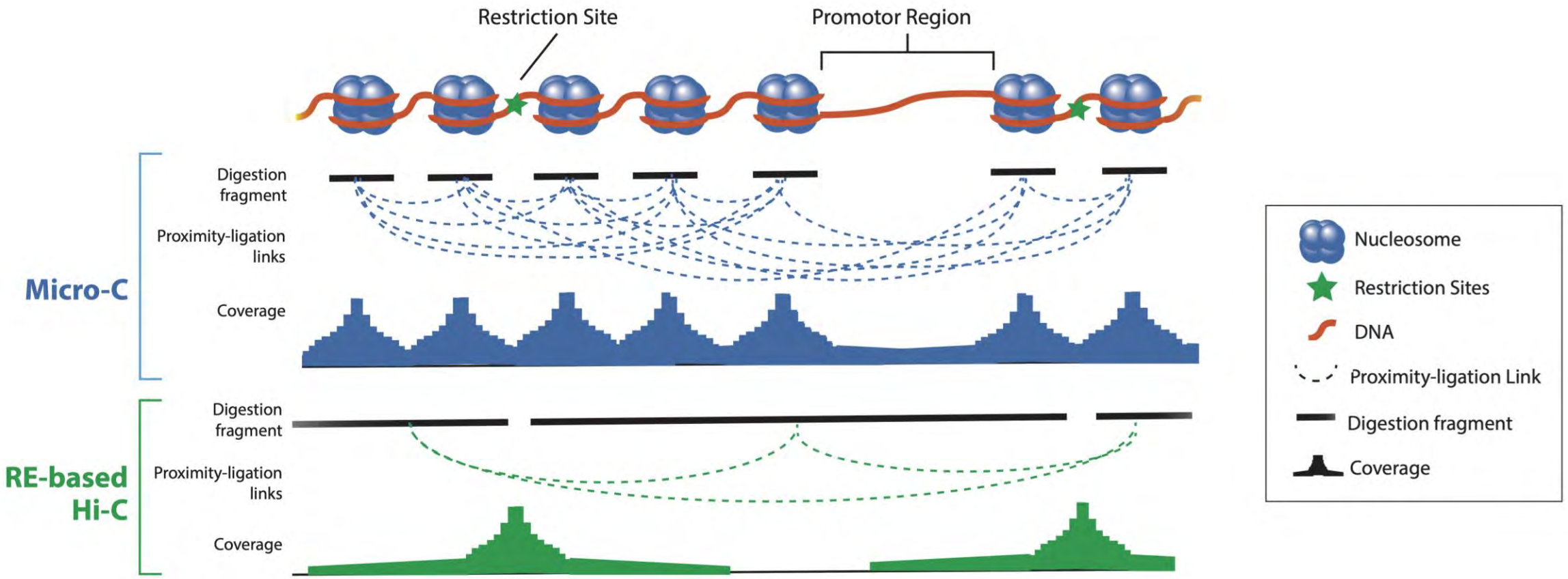
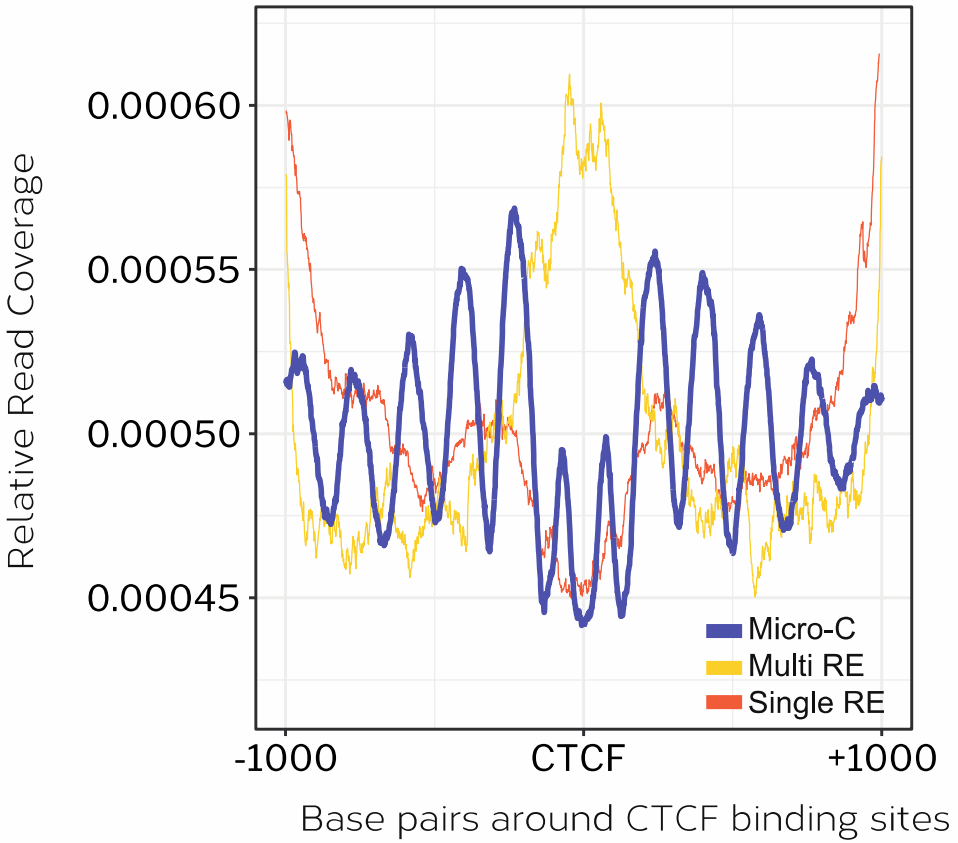
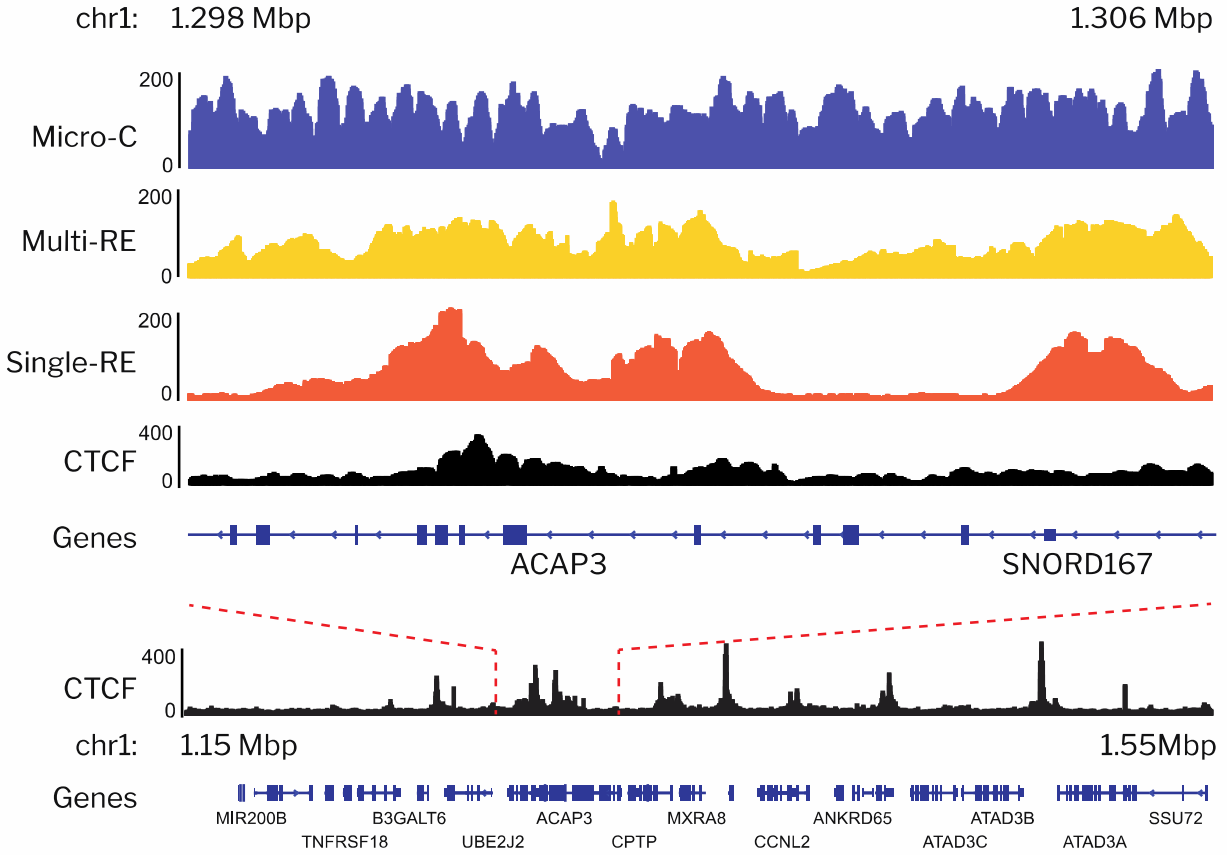
Micro-C Breaks the Resolution Barrier

Micro-C Offers Enhanced Resolution Down To The Nucleosome Level

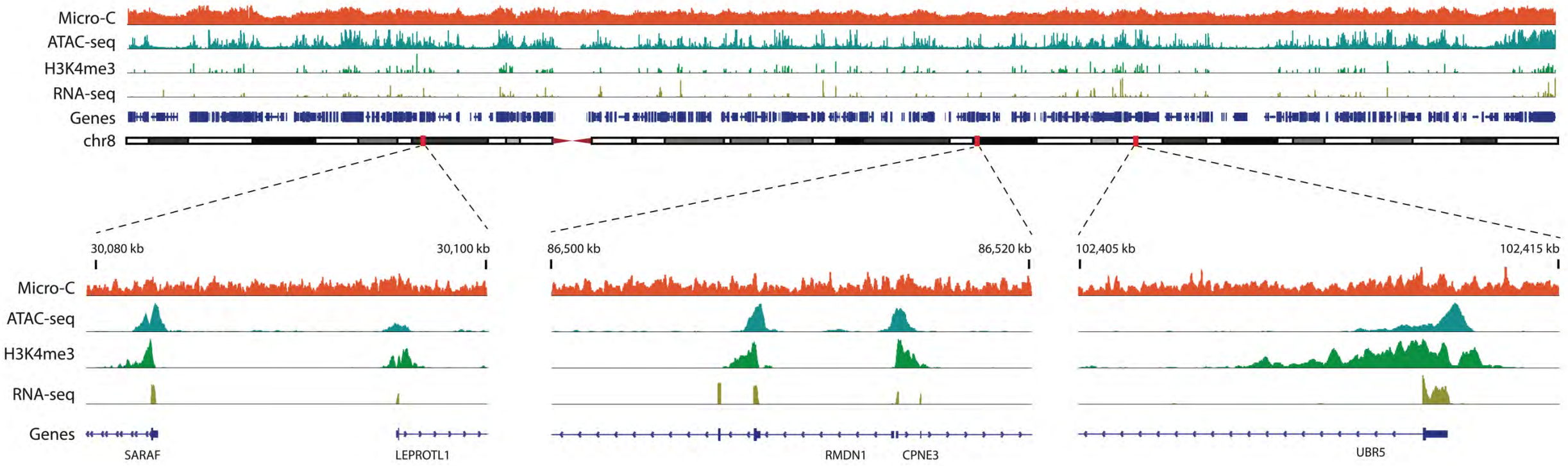


Capture nucleosome position during proximity-ligation



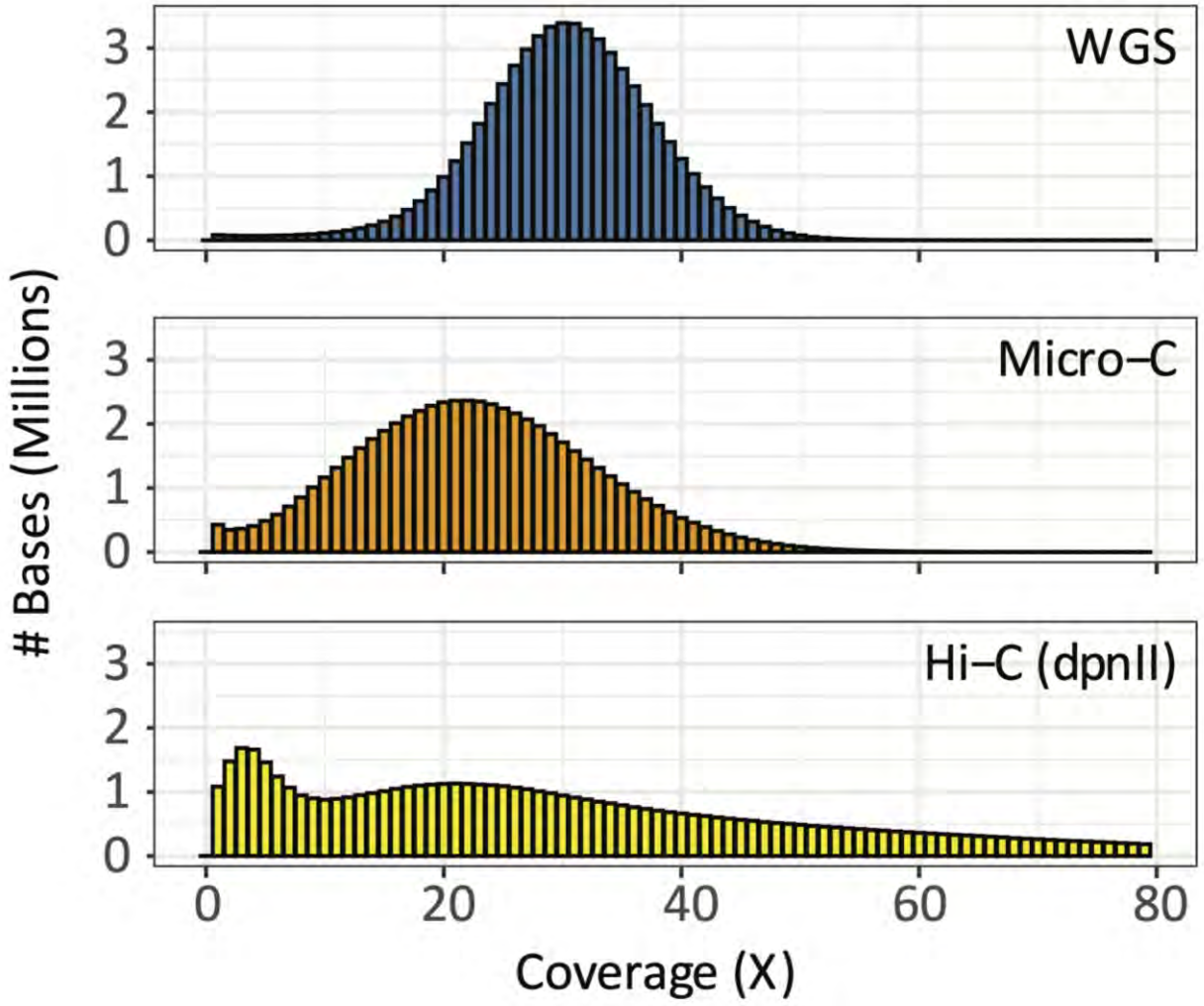


Micro-C Maintains Coverage Over Nucleosome-Free Regions



Micro-C Genomic Coverage is More Uniform Than RE-based Hi-C

- Micro-C has a normal distribution
- Wider histogram, shifted slightly to left compared to WGS
- This is because we sacrifice some of the linker DNA during digestion

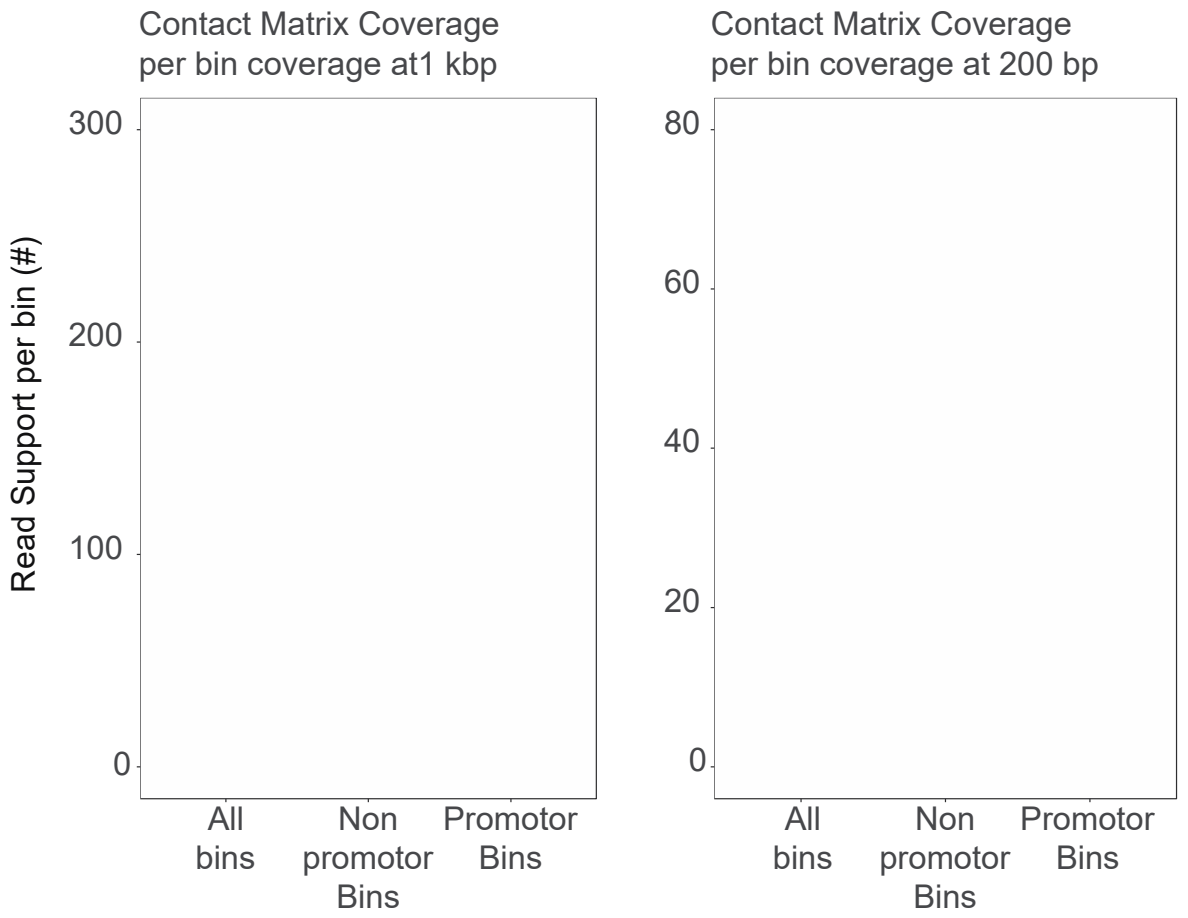


Micro-C Bins Maintain Coverage at Promotor Regions

When building Hi-C contact matrices, typically data binned into a resolution that is supported by:

- Fragment size
- Read-support

Micro-C bins maintain coverage over nucleosome deficient regions, like promotors



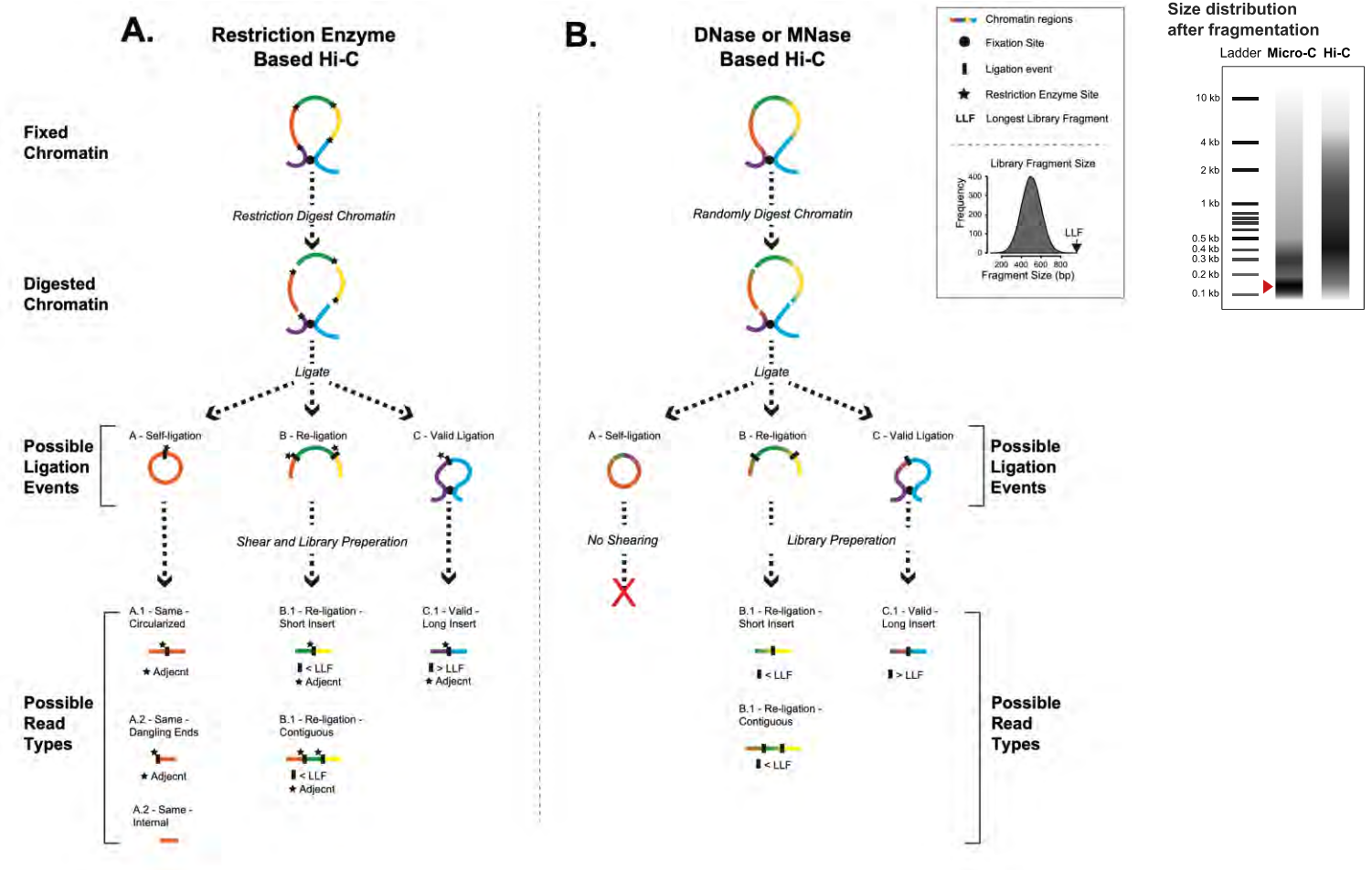
Micro-C Smaller Fragment Size Increase the Amount of Useable Reads

Smaller Micro-C fragments remove the need for sheering during library conversion

Eliminates self-ligation events from the data

Dual-cross linker approach reduces the amount of re-ligation events

The result is a library enriched in useable Hi-C Reads and and increased signal-to-noise ratio



Improved Signal-To-Noise Enables Superior Detection Of Higher-Order Chromatin Features

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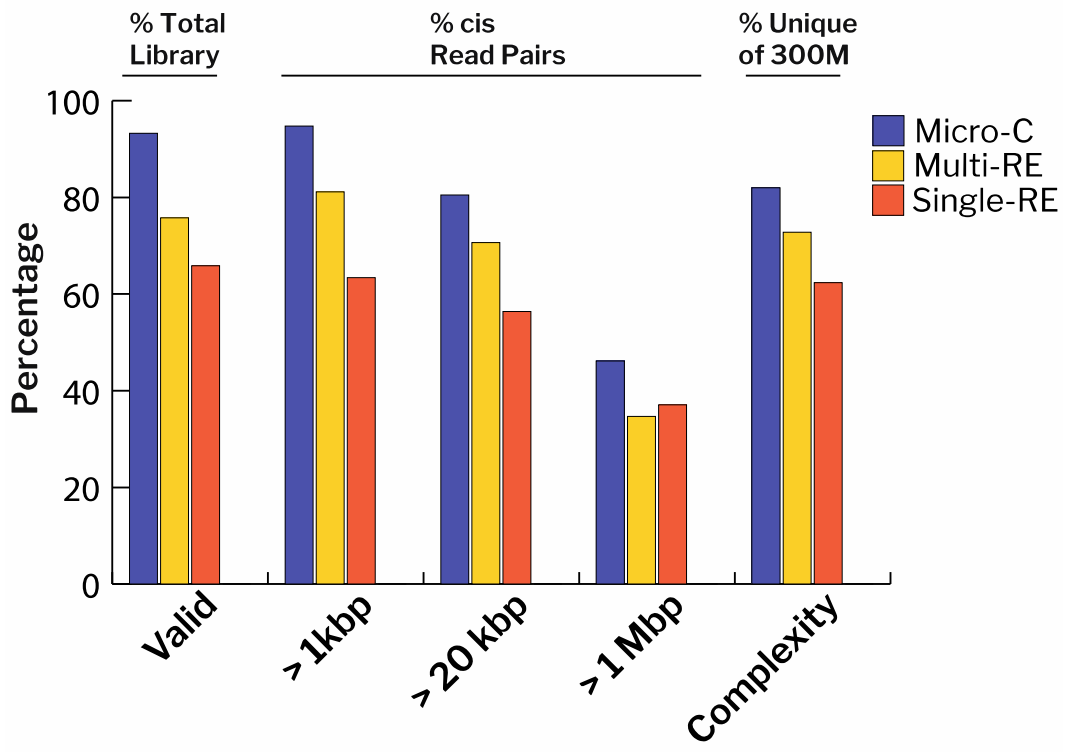
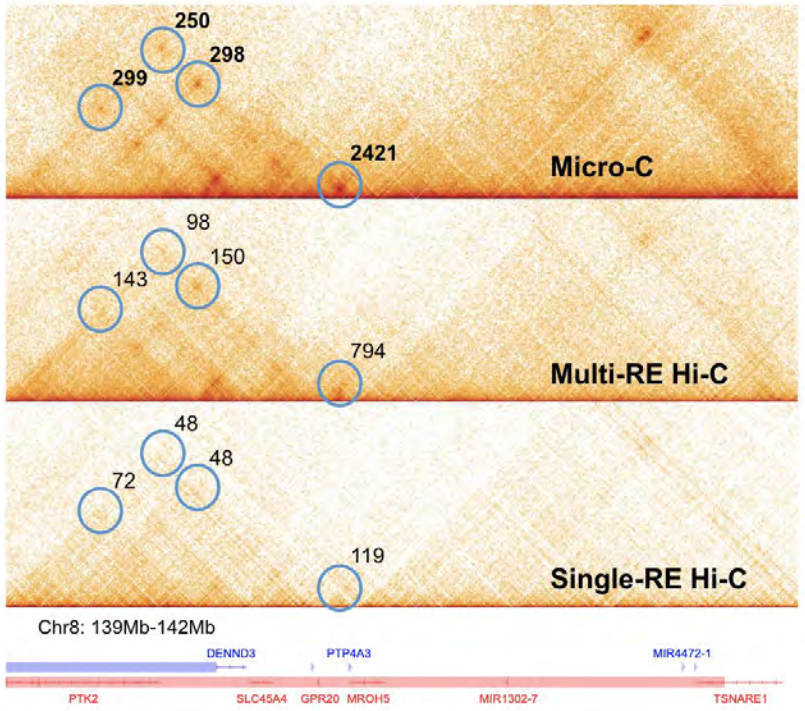
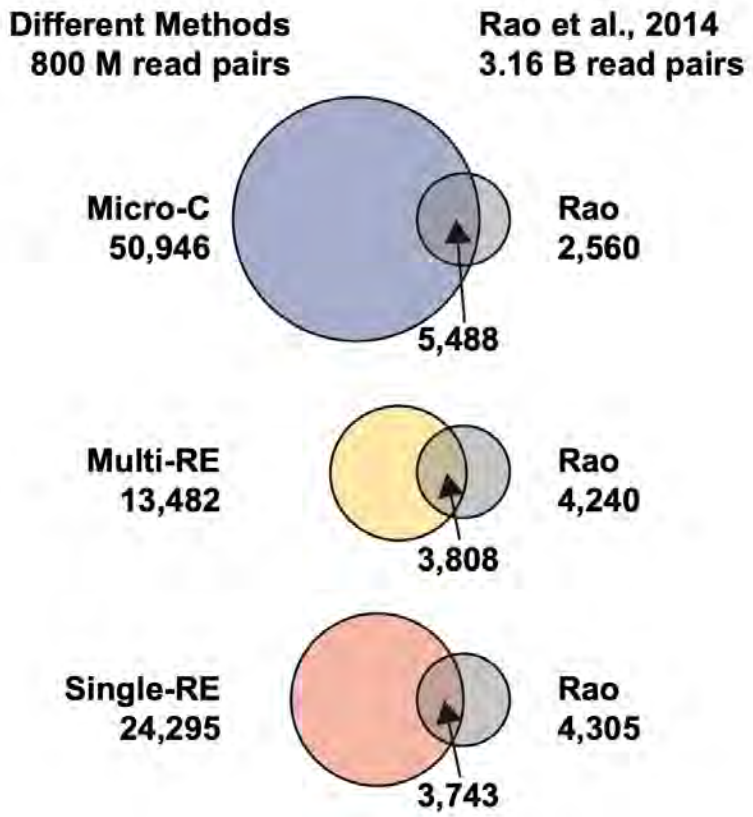
Library Stats

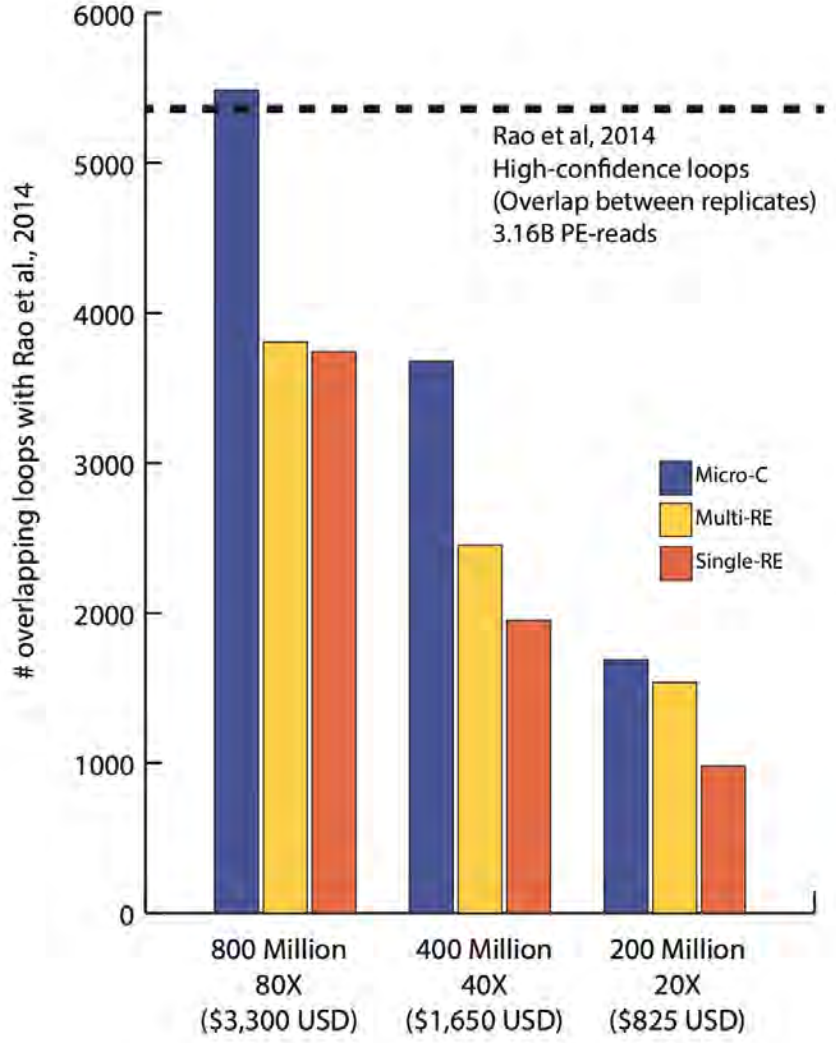


4 kbp Contact Matrices From GM12878 Cell Lines Each Matrix Normalized To 800 M Read Depth



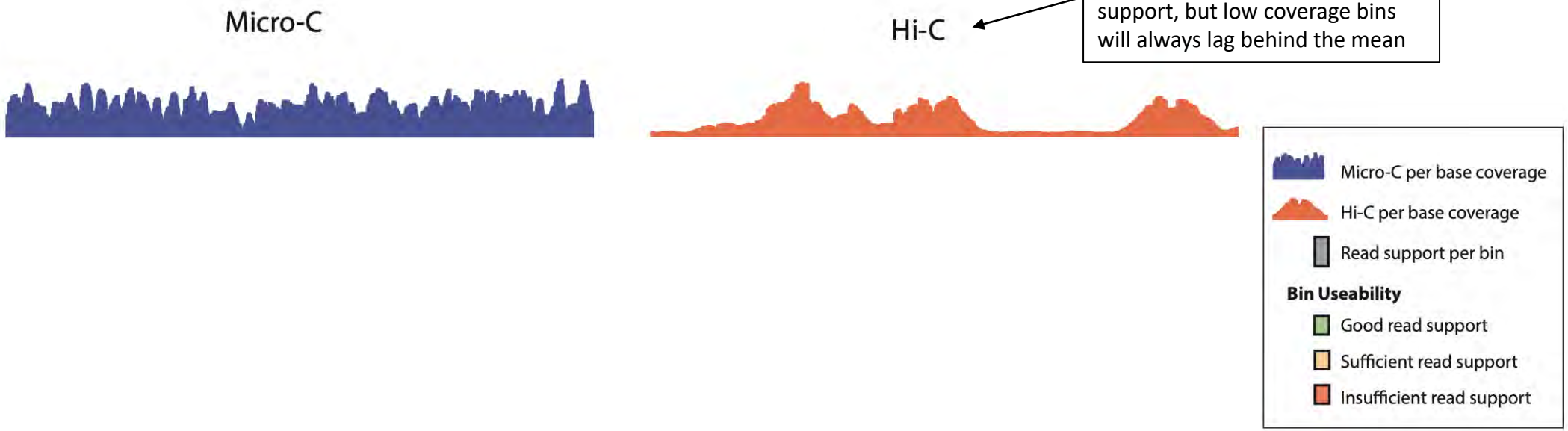
Micro-C robustly detects chromatin loop structures at a fraction of the sequencing



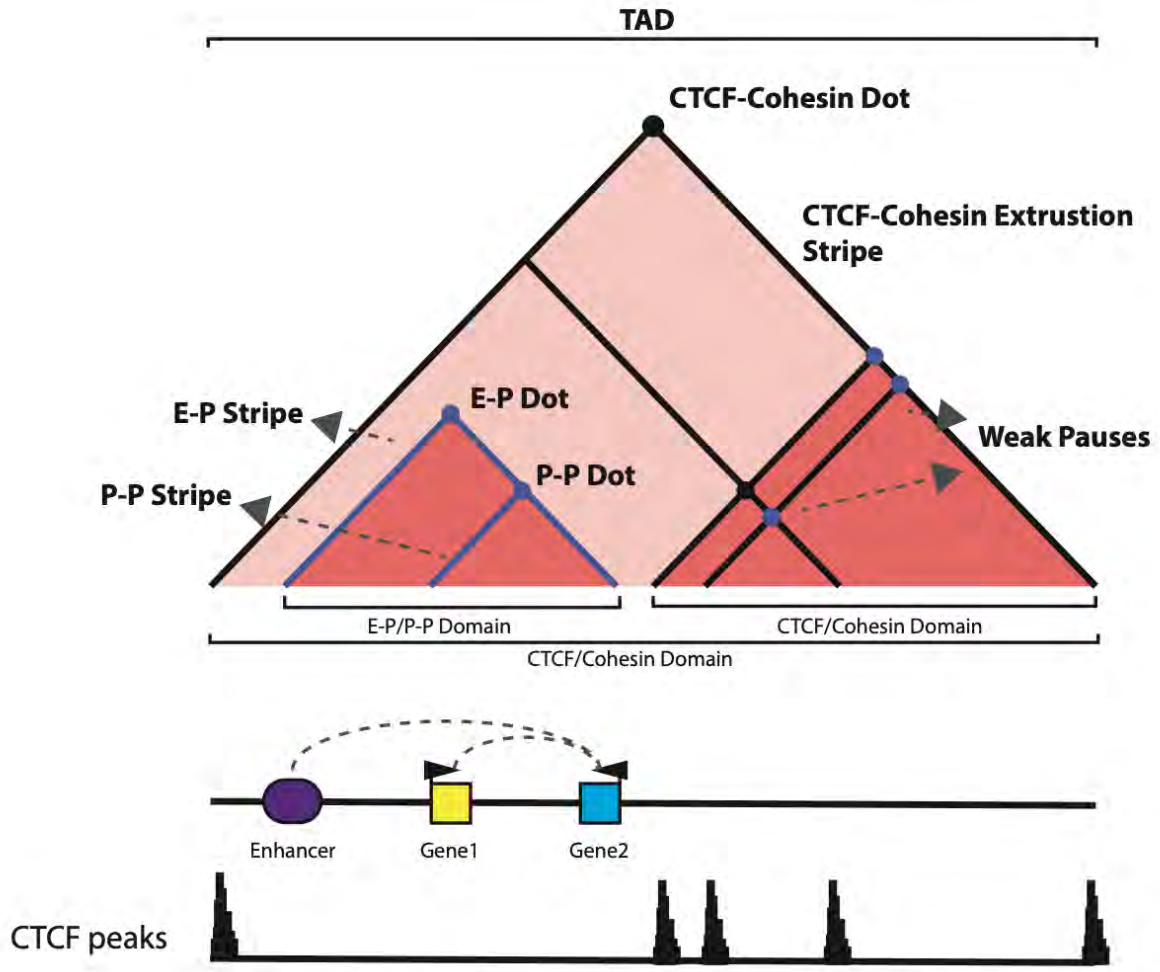


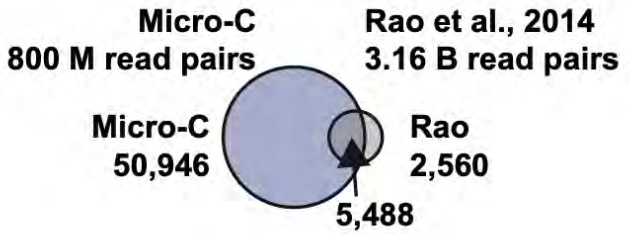
Micro-C Contact Matrices are Superior Even at 1 kb Resolution

Would need to sequence 35-50% more to make provide more read support, but low coverage bins will always lag behind the mean



Superior resolution reveals the anatomy of a TAD





	Micro-C	Hi-C
A/B Compartments	✔	✔
TADs	✔	✔
CTCF-Loop Dots	✔	✔
CTCF-Stripe	✔	✔
Extrusion Weak Pause	✔	
E-E/E-P Dots	✔	
E-E/E-P Stripe	✔	

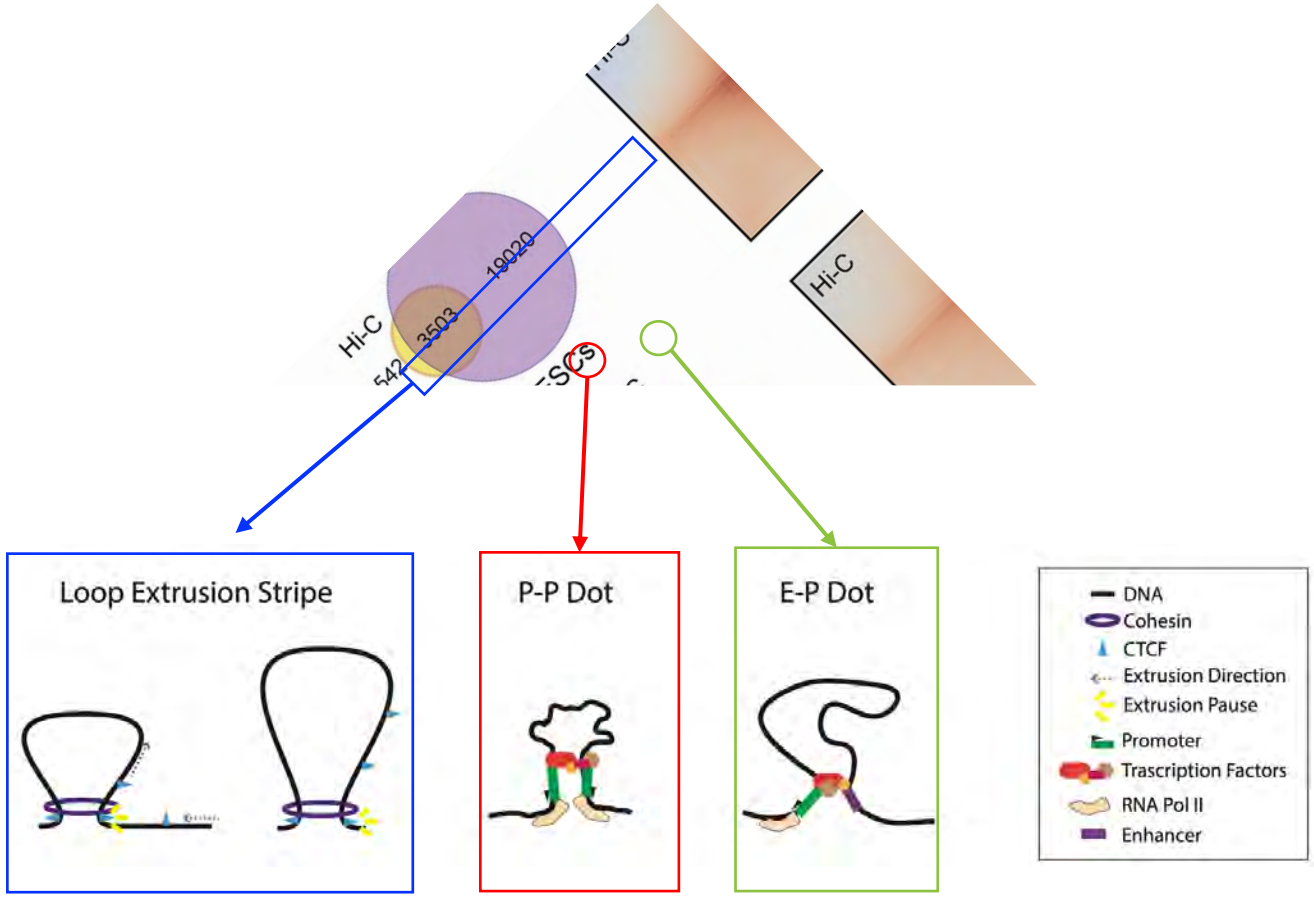
Sub-TAD Conformation Features

The improved contact matrices illuminate fine-scale conformation features including:

- Loop Extrusion
- Promoter-Promoter interactions
- Enhancer-Promoter interactions

When combined with primary feature epigenetic locations, such as methylation or protein binding new models of chromatin dynamics can be described

Sub-TAD chromatin features



Dovetail™ Micro-C: Nucleosome Positioning Generates The Highest Resolution View of Conformation

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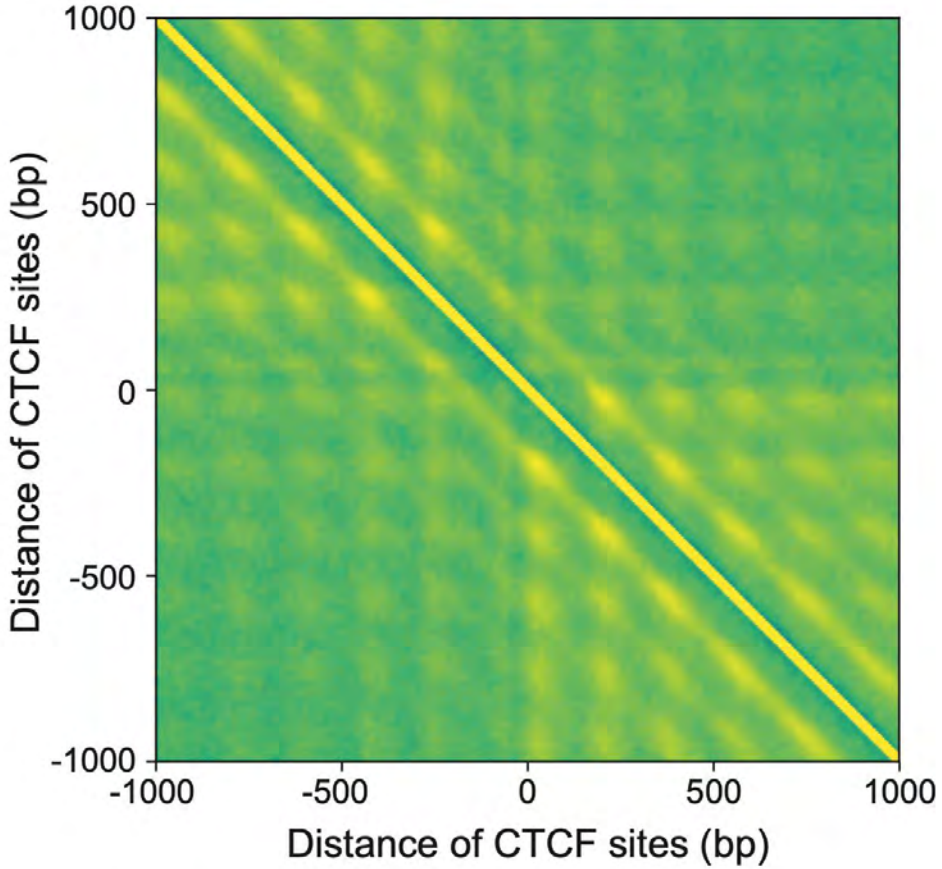
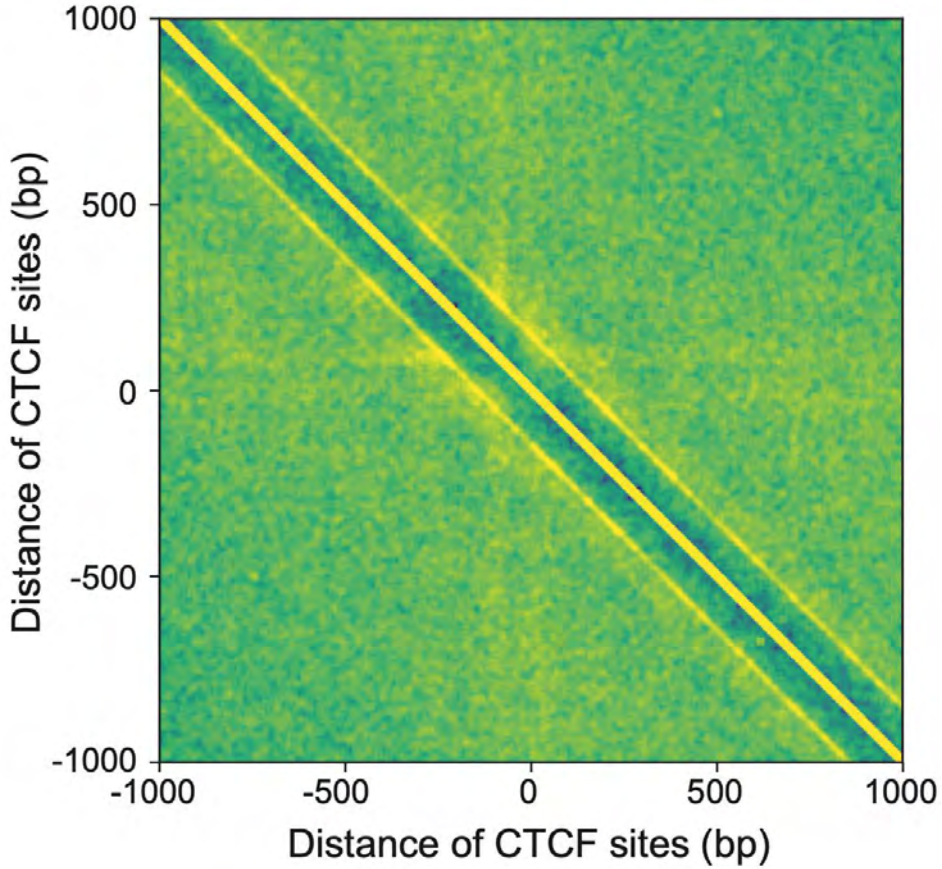
Dovetail™ Micro-C

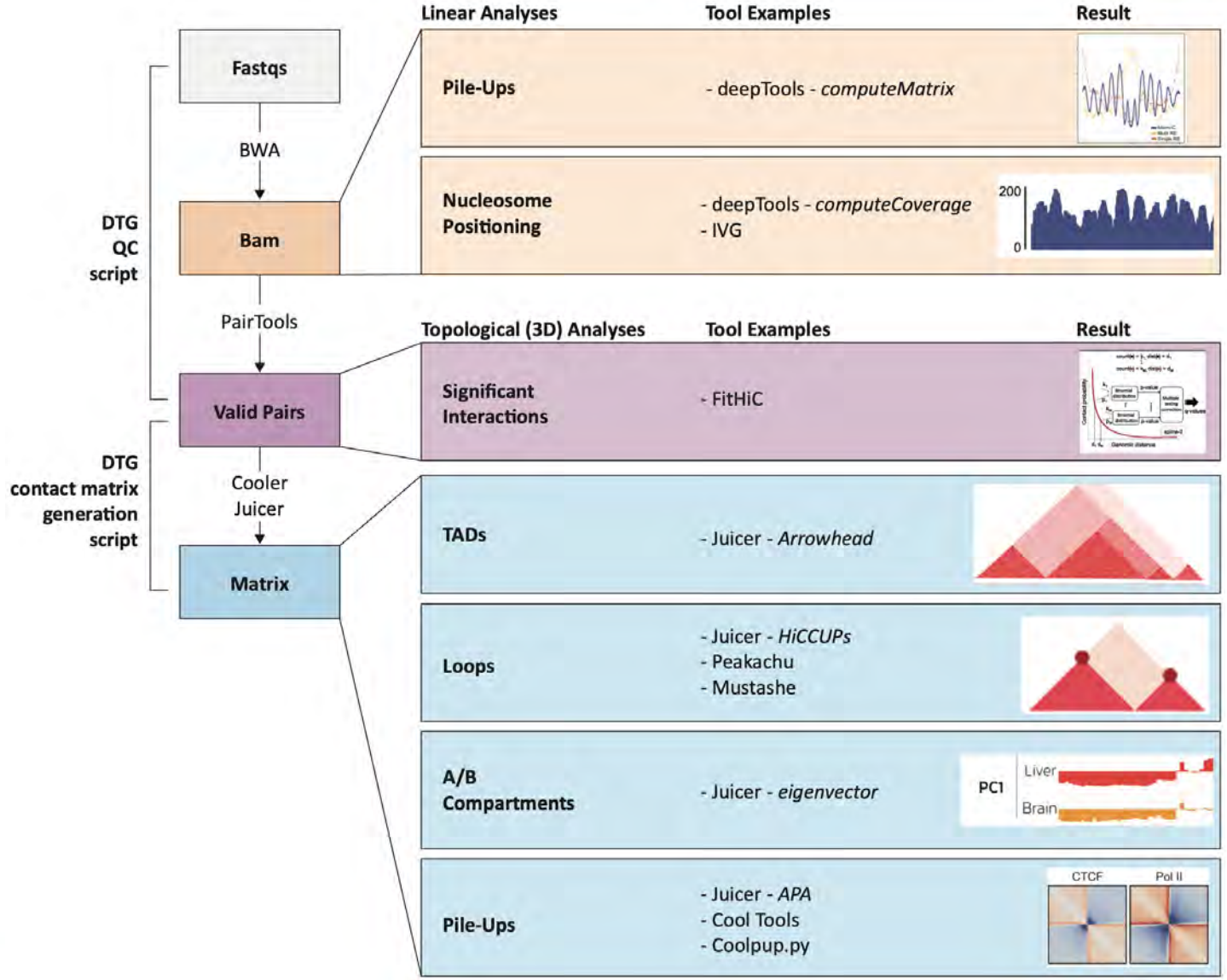


Multi-RE Hi-C



General Analytical Workflow

- Both Linear and 3D analyses can be preformed
- The key files that integrate into analyses are
  - Alignment (,bam)
  - Valid Pairs (.txt)
  - Matrix (.cool/.hic)
- All of these files are generated from the DTG Github scripts
- These are just a few examples of tools that can be used to process Micro-C data



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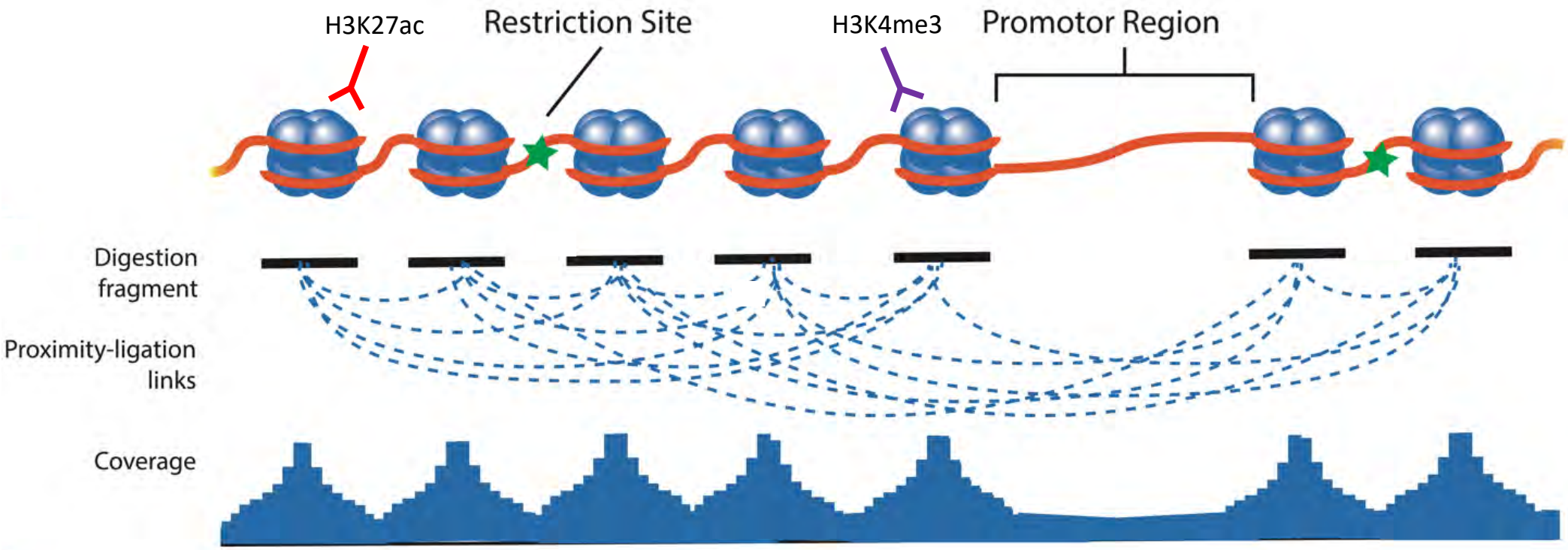
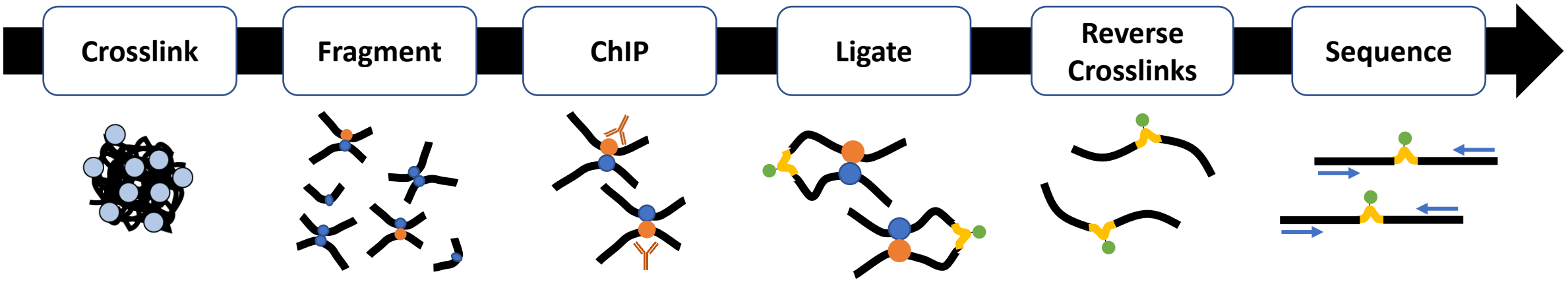
Further leveraging Micro-C in Epigenetics

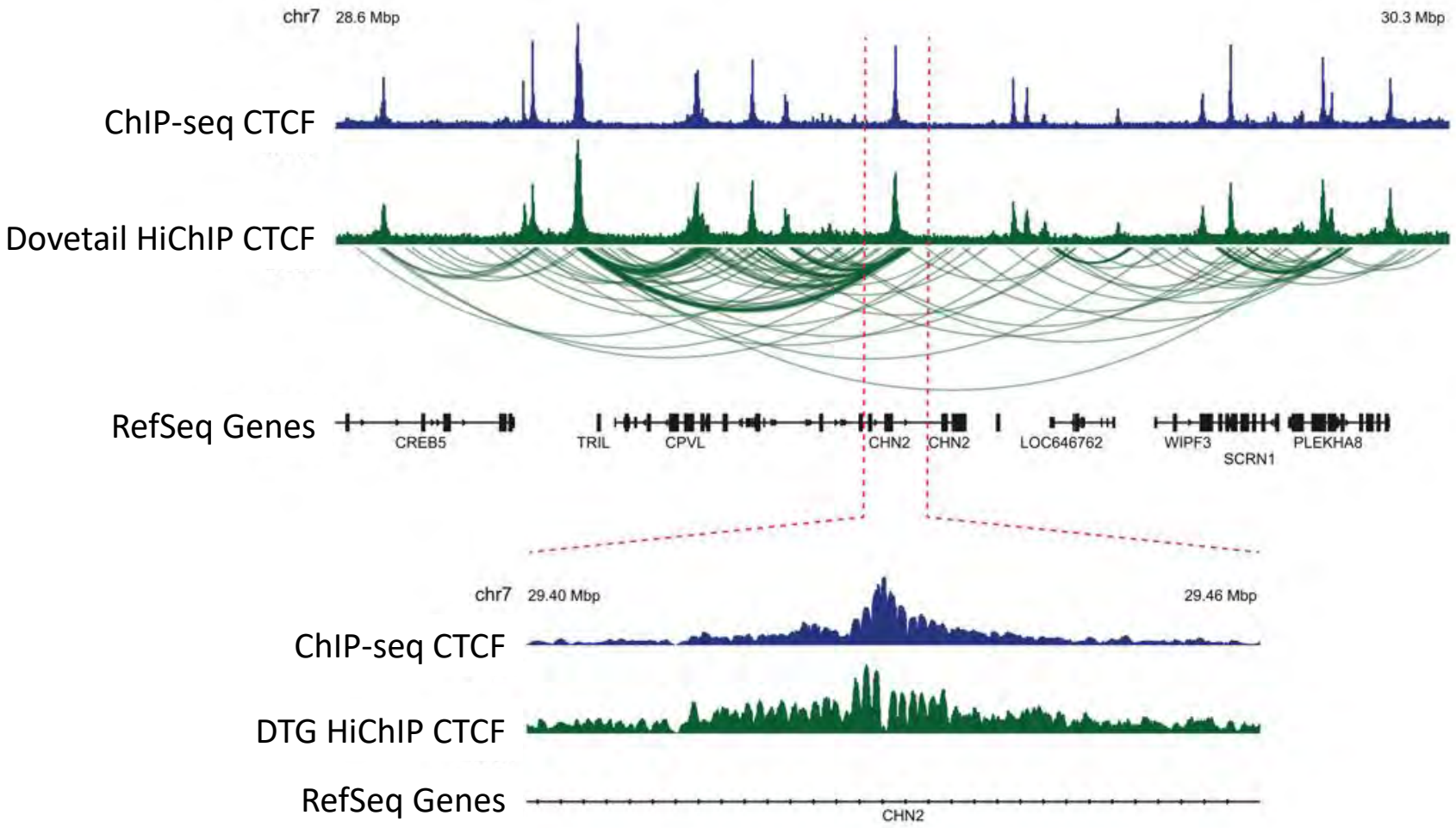
Protein-directed Conformation



Dovetail™ HiChIP Workflow Combines Hi-C and ChIP-seq

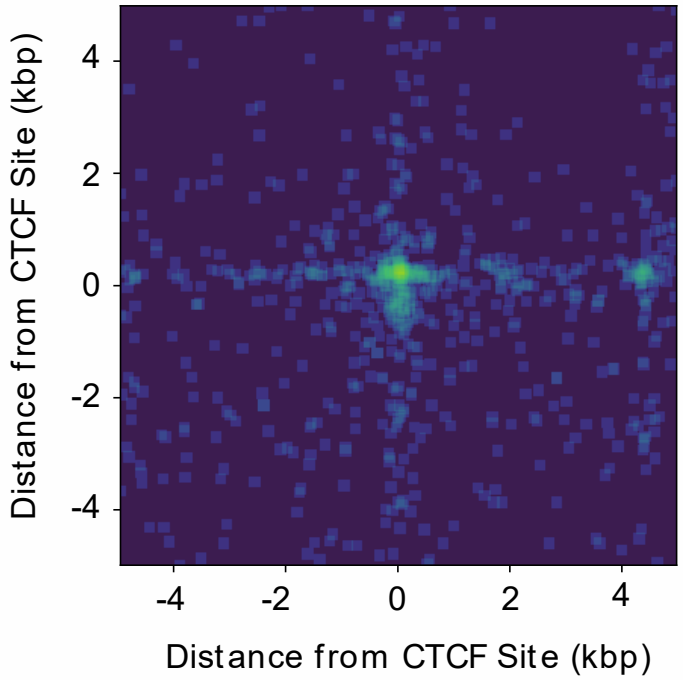


Capture ChIP-seq Data & Hi-C Long-range Information In A Single Library



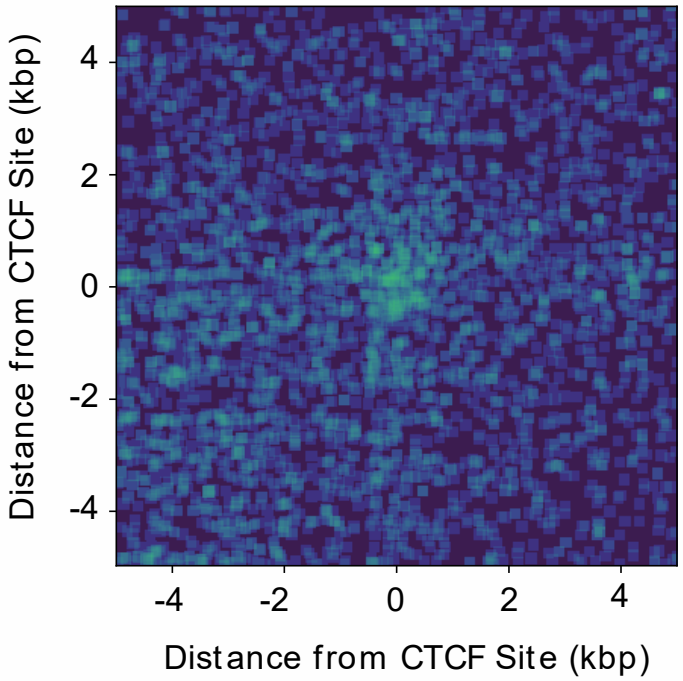
Increased signal to noise ration decreases the cost to assess topology at a region of interest

HiChIP
150M read pairs



Signal to noise ratio = 2.5

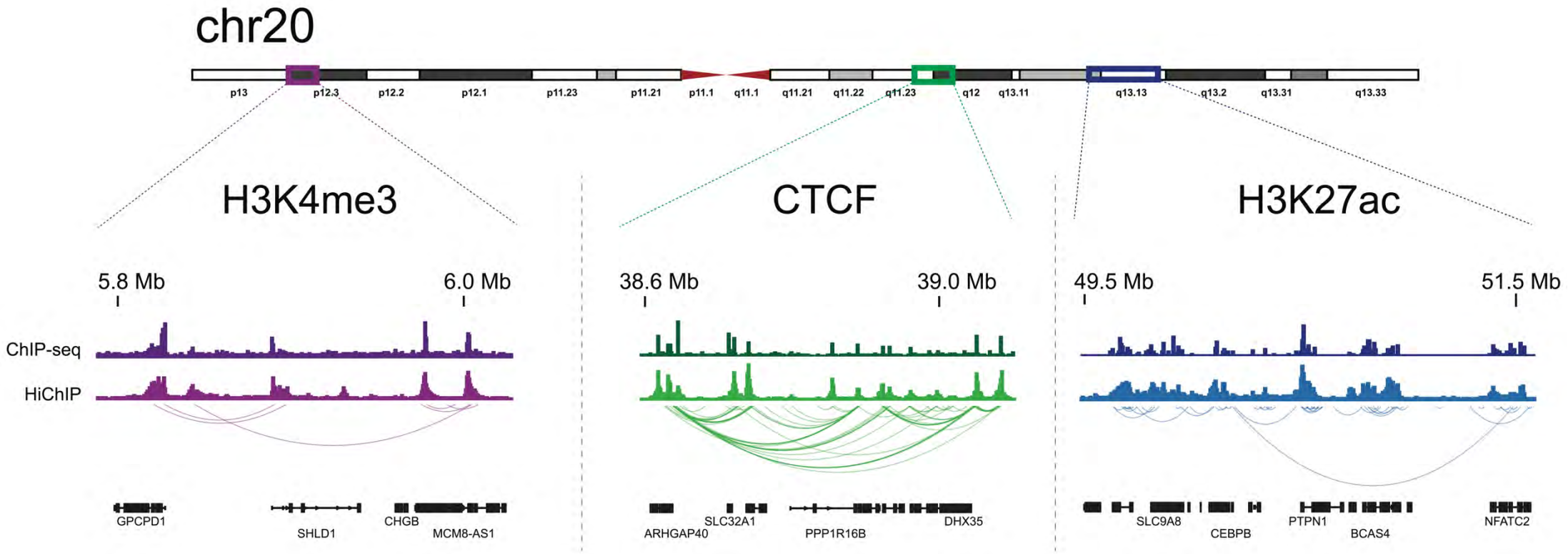
Multi-RE Hi-C
800M read pairs



Signal to noise ratio = 0.9

Signal to noise ration is calculated by: the mean coverage of the top 25 percentile over target sites divided by the coverage (top 25 percentile) over non-target sites normalized to IgG

A wide-range of validated antibodies



How is Dovetail HiChIP different from other HiChIP assays?

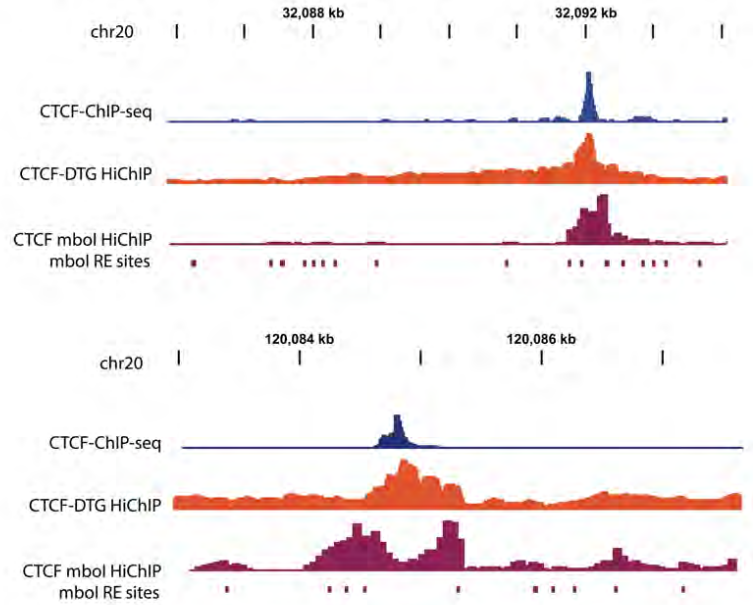
MNase provides a more biologically relevant view of chromatin interactions

RE-based HiChIP can distort or even miss ChIP signals

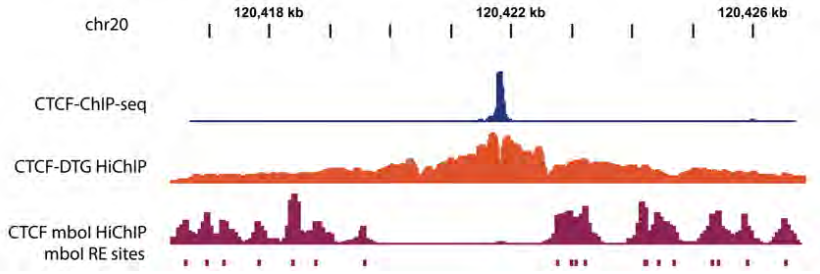
RE-based HiChIP complicates computation because the signal needs to be corrected for RE-pile up

Dovetail's approach to HiChIP asks a more direct AND biologically relevant question of chromatin architecture

Peak Distortion through REs



Loss of peak signal due to REs



Easy Adoption With Pre-Validated Antibodies

Dovetail™ HiChIP Validated Antibody	Supplier	Part Number
IgG	Cell Signaling	2729
CTCF	Cell Signaling	3418
H3K4ac	Active Motif	39381
H3K4me3	Cell Signaling	9751
H3K14ac	Cell Signaling	7627
H3K27ac	Cell Signaling	8173
H3K27me3	Cell Signaling	9733
H3K36me3	Cell Signaling	4909
SMC3	Abcam	ab9263
Oct4	Cell Signaling	2890
Sox2	Cell Signaling	23064
Klf4	Abcam	ab106629
Nanog	Abcam	ab21624
PolII	Active Motif	61667

Thank You & Questions