# Long Range Interaction Analysis in the Age of ENCODE

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Gerstein and Snyder Labs
June 22, 2011

# Outline

- Brief overview of work since last group meeting

- Review of current trends in long-range interactions
  - Chromatin Conformation Capture Family
  - Chromatin Interaction Analysis by Paired-End diTag Sequencing (ChIA-PET)

- Selected Results from a pilot ChIA-PET RNAPII experiment

- Discussion of possible ChIP-Seq tool set

# Selected Papers

- modENCODE
  - Analysis of the C. elegans genome (Gerstein et al)
  - Characterization of Pol II binding and gene expression

- A Genome-Wide View of Chromatin Remodeling
  - Euskirchen and Auerbach et al., PLoS Genetics
  - Atypical trancription factors, data quality and integration, new techniques and approaches

- Characterization of Long-Range Interactions in Multiple Cell Lines Using ChIA-PET
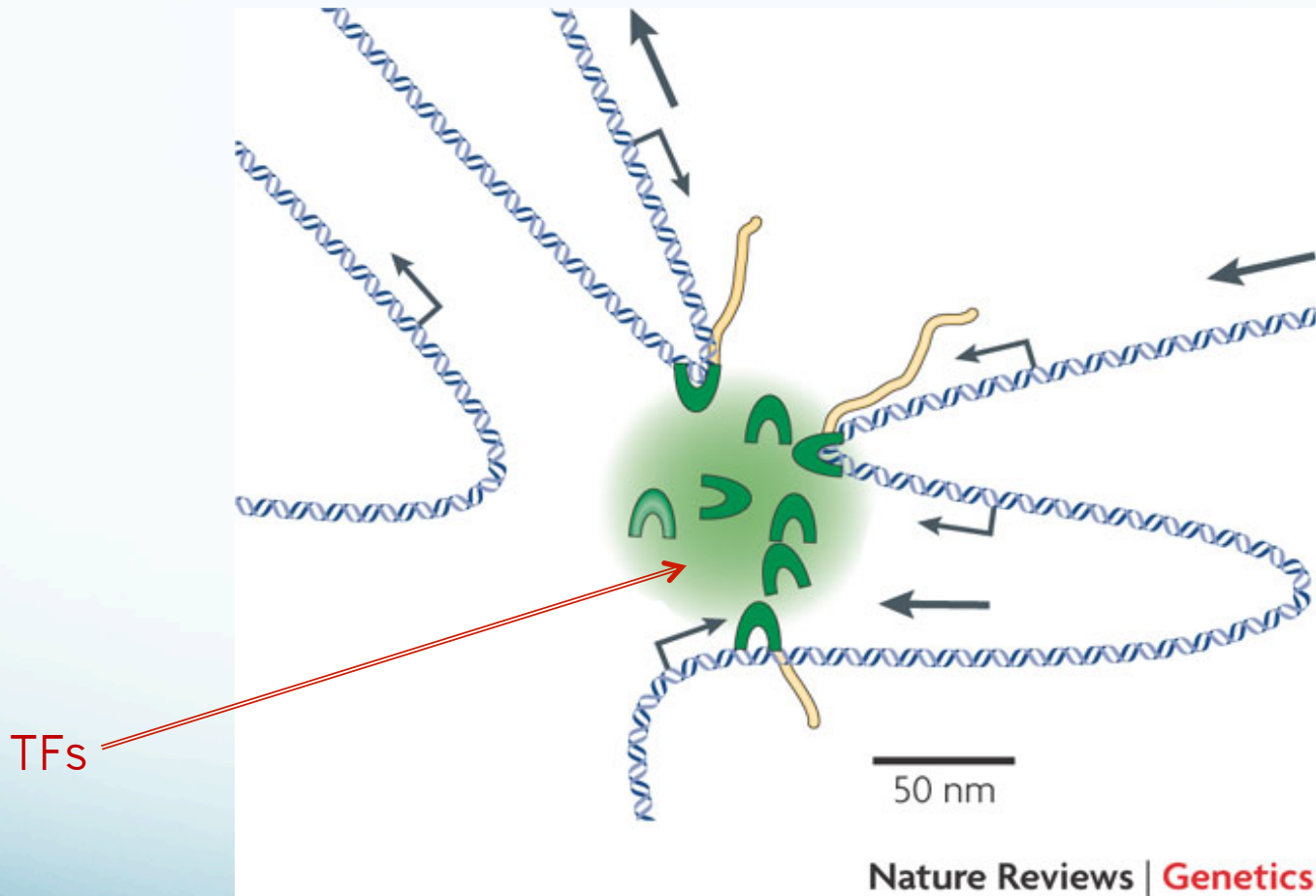  - Li, Ruan, and Auerbach, et al.
  - In revision

# Before We Begin…

- Disclaimers
  - I am not an experimentalist
  - LRI methods are still under active development
  - This is a pilot project

# LRI: The Basics

# Genomic Life in 1-D…

# And Now in 3-D (with Looping)



TFs

50 nm

Nature Reviews | Genetics

Heidi Sutherland & Wendy A. Bickmore. Transcription factories: gene expression in unions? Nature Reviews Genetics 10, 457-466 (July 2009).

# The Case for Transcription Factories

- Thought to be over ~8,000 RNAPII and ~2,000 RNAPIII factories in the nucleoplasm
  - Erythroid cells. Fewer in tissues.

- More genes need to be transcribed at a faster rate than physically possible
  - Bind and slide, random binding, etc.
  - Instead, bring the DNA to the TFs

- Confirmed by FISH

- Nucleolus and RNAPI

# Targeted Recruitment

Isolated by ChIP
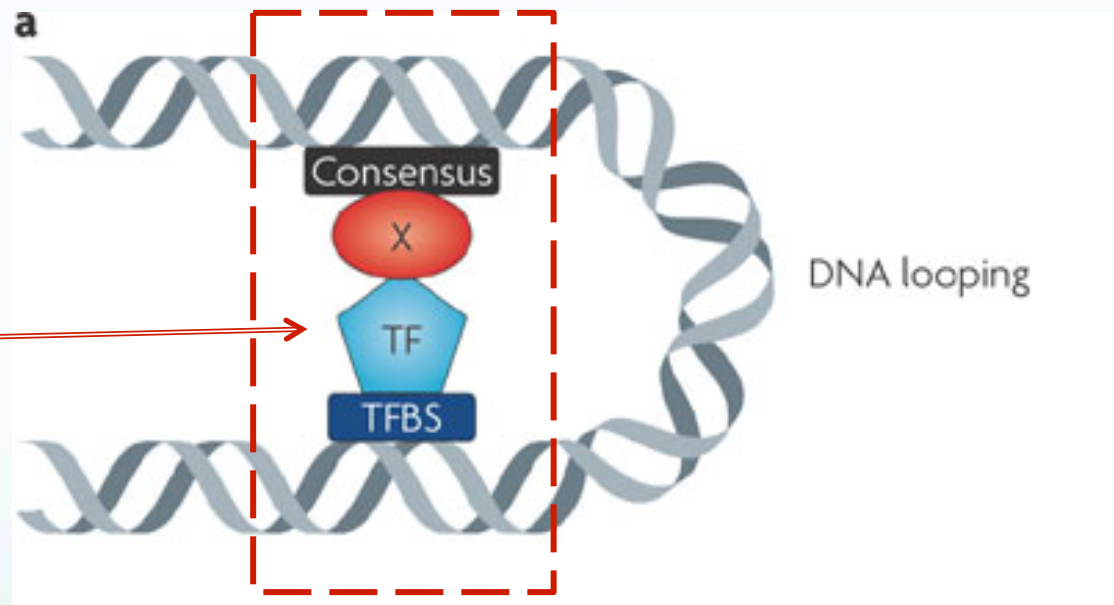
PJ Farnham, Nature Reviews Genetics, 2009

# Current Methods in LRI Analysis

- Chromatin Conformation Capture
  - UMass
  - Advantage: Can be targeted to a specific locus or extended to all chromosomal contacts genome-wide
  - Disadvantage: Can't isolate by site-specific properties. A lot of primer design. Noise issues.

- ChIA-PET
  - Genome Institute of Singapore
  - Advantage: One can get a complete set of all interactions involving a particular TF.
  - Disadvantage: Interaction catalog is not generalized, no obvious way to filter noise across chromosomes.

# Chromatin Conformation Capture Family

11
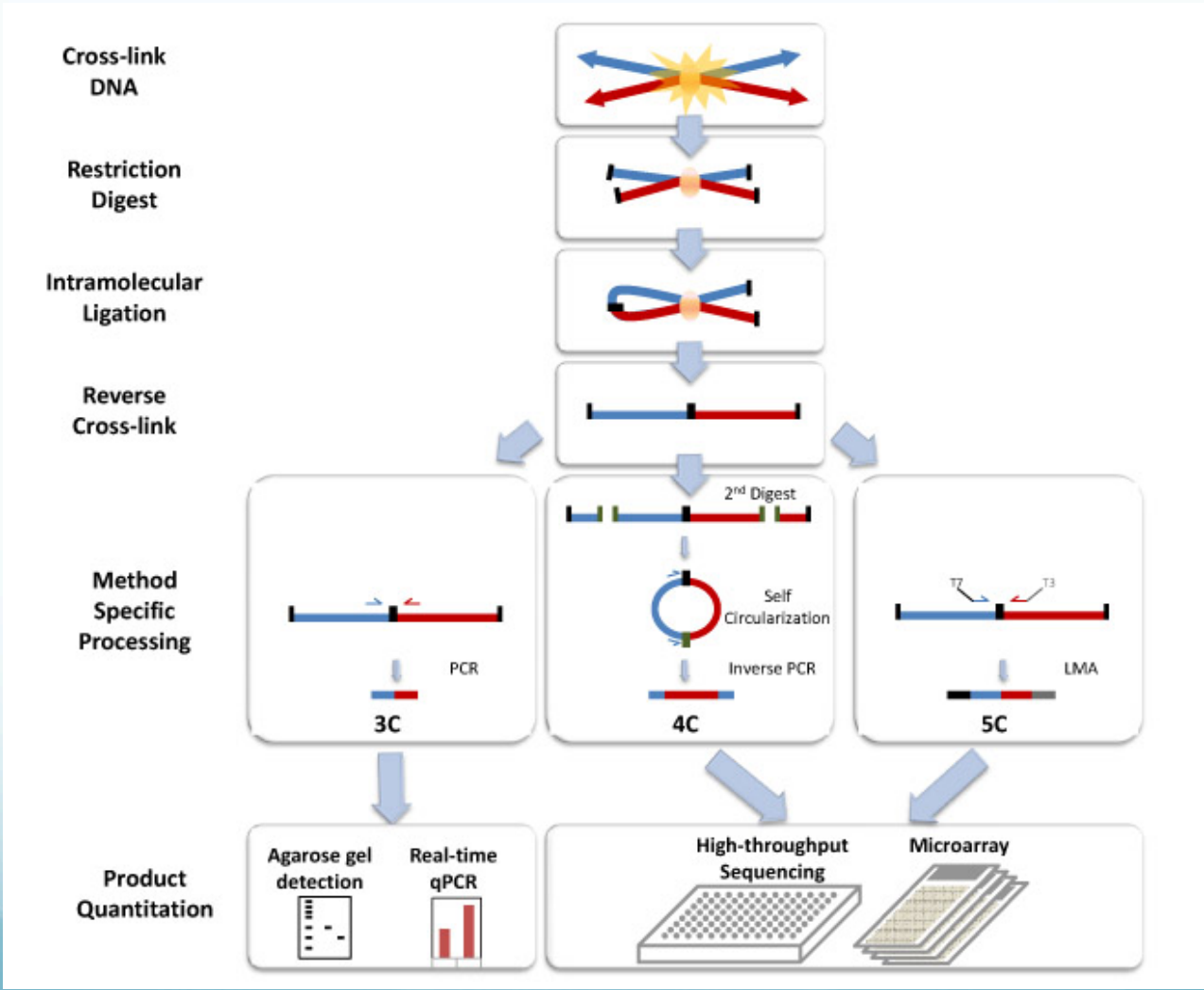
# 5c Data on UCSC

- UMass
  - 5c interactions between ENCODE Pilot Regions
  - GM12878, H1-hESC, HeLa-S3, and K562
  - Replicates Not Combined

- UW
  - A bunch of lines
  - Mainly focuses on Myc
  - Replicates Not Combined

# What You Will See on UCSC

and **Description:**    contains [ ]

and **Group:**    is [ Any ]

and **Data Format:**    is [ Any ]

*ENCODE terms*

(+) and [ Experiment (Assay) type ] is among [ 5C ]

(+) and [ Antibody or target protein ] is among [ Any ]   [Antibody or target protein](#)

(search) (clear) (cancel)

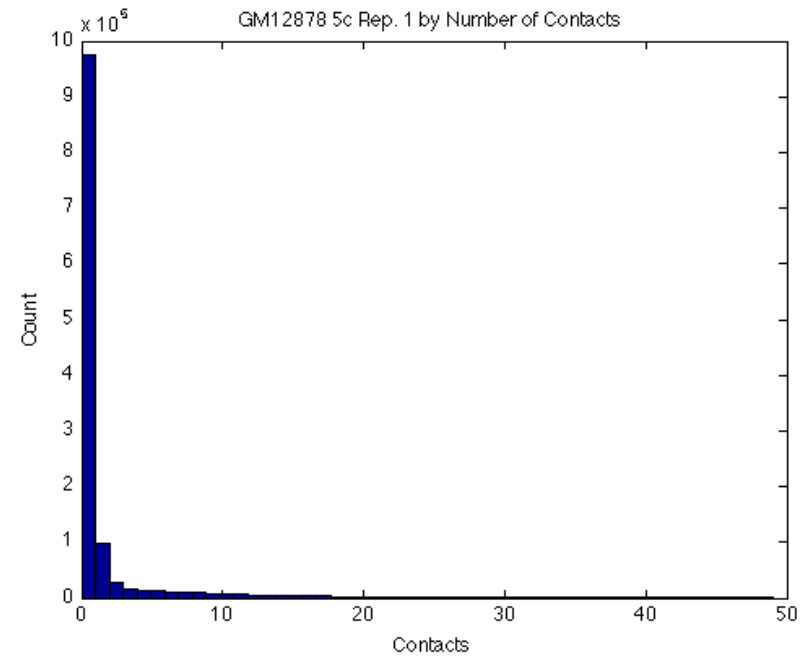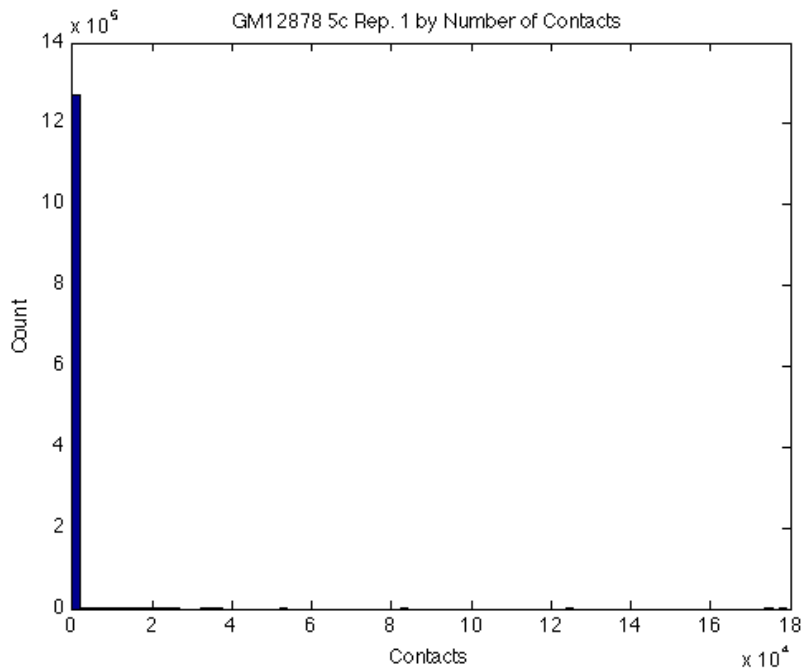| 44 files | Principal Investigator on grant↓1 | Lab producing data↓2 | View - Peaks or Signals↓3 | Cell, tissue or DNA sample↓4 | Date restrictions end↓5 | Replicate number | ENCODE Data Freeze | Date submitted to UCSC | Submission ID | Genomic region(s) | Size | File Type | Additional Details |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Download | Dekker | UMass-Dekker | Matrix | GM12878 | 2011-10-25 | 1 | ENCODE Jan 2011 Freeze | 2011-01-25 | 3488 | | 1.0 MB download | | dataType=5C; |
| Download | Dekker | UMass-Dekker | Matrix | GM12878 | 2011-10-25 | 2 | ENCODE Jan 2011 Freeze | 2011-01-25 | 3488 | | 851 KB download | | dataType=5C; |
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| Download | Dekker | UMass-Dekker | Matrix | H1-hESC | 2011-10-25 | 2 | ENCODE Jan 2011 Freeze | 2011-01-25 | 3488 | | 513 KB download | | dataType=5C; |
| Download | Dekker | UMass-Dekker | Matrix | HeLa-S3 | 2011-10-25 | 1 | ENCODE Jan 2011 Freeze | 2011-01-25 | 3488 | | 841 KB download | | dataType=5C; |
| Download | Dekker | UMass-Dekker | Matrix | HeLa-S3 | 2011-10-25 | 2 | ENCODE Jan 2011 Freeze | 2011-01-25 | 3488 | | 788 KB download | | dataType=5C; |
| Download | Dekker | UMass-Dekker | Matrix | K562 | 2011-10-25 | 1 | ENCODE Jan 2011 Freeze | 2011-01-25 | 3488 | | 1.0 MB download | | dataType=5C; |
| Download | Dekker | UMass-Dekker | Matrix | K562 | 2011-10-25 | 2 | ENCODE Jan 2011 Freeze | 2011-01-25 | 3488 | | 1.0 MB download | | dataType=5C; |
| Download | Stam | UW | Matrix | BJ | 2011-12-08 | 1 | post ENCODE Jan 2011 Freeze | 2011-03-09 | 3813 | UW-5C-Gene-Regions | 108 KB download | | dataType=5C; |
| Download | Stam | UW | Matrix | BJ | 2011-12-08 | 2 | post ENCODE Jan 2011 Freeze | 2011-03-09 | 3813 | UW-5C-Gene-Regions | 116 KB download | | dataType=5C; |
| Download | Stam | UW | Matrix | Caco-2 | 2011-12-08 | 1 | post ENCODE Jan 2011 Freeze | 2011-03-09 | 3813 | UW-5C-Myc-Region | 64 KB download | | dataType=5C; |
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| Download | Stam | UW | Matrix | GM12878 | 2011-12-08 | 1 | post ENCODE Jan 2011 Freeze | 2011-03-09 | 3813 | UW-5C-Myc-Region | 59 KB download | | dataType=5C; |
| Download | Stam | UW | Matrix | GM12878 | 2011-12-08 | 2 | post ENCODE Jan 2011 Freeze | 2011-03-09 | 3813 | UW-5C-Myc-Region | 60 KB download | | dataType=5C; |
| Download | Stam | UW | Matrix | HepG2 | 2011-12-08 | 1 | post ENCODE Jan 2011 Freeze | 2011-03-09 | 3813 | UW-5C-Gene-Regions | 158 KB download | | dataType=5C; |
| Download | Stam | UW | Matrix | HepG2 | 2011-12-08 | 2 | post ENCODE Jan 2011 Freeze | 2011-03-09 | 3813 | UW-5C-Gene-Regions | 116 KB download | | dataType=5C; |
| Download | Stam | UW | Matrix | K562 | 2011-12-08 | 1 | post ENCODE Jan 2011 Freeze | 2011-03-09 | 3813 | UW-5C-Myc-Region | 51 KB download | | dataType=5C; |
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| Download | Stam | UW | RawData | BJ | 2011-12-08 | 2 | post ENCODE Jan 2011 Freeze | 2011-03-09 | 3813 | UW-5C-Gene-Regions | 1.6 GB | fastq | dataType=5C; |
| Download | Stam | UW | RawData | Caco-2 | 2011-12-08 | 1 | post ENCODE Jan 2011 Freeze | 2011-03-09 | 3813 | UW-5C-Myc-Region | 3.2 GB | fastq | dataType=5C; |
| Download | Stam | UW | RawData | Caco-2 | 2011-12-08 | 2 | post ENCODE Jan 2011 Freeze | 2011-03-09 | 3813 | UW-5C-Myc-Region | 1.8 GB | fastq | dataType=5C; |

# What is the Matrix?

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#my5C - http://my5C.umassmed.edu
#15:39:40, Tue Jan 18, 2011
#my5C temp file - 500_c516a3583d1358c541f06009658b6192
#ALLxALL MATRIX
#primerpool - 966_ENM
#result - 1774_ENM1-GM12878
#
```

| | 5C_299_ENm002_REV_1\|hg19\|chr5:131256415-131258341 | 5C_299_ENn | 5C_299_ENn | 5C_299_ENn | 5C_299_ENn | 5C_299_ENn | 5C_299_ENn | 5C_299_ENn |
|---|---|---|---|---|---|---|---|---|
| 5C_299_ENm002_FOR_2\|hg19\|chr5:131258341-131260605 | 727 | 426 | 154 | 17 | 79 | 68 | 173 | 99 |
| 5C_299_ENm002_FOR_5\|hg19\|chr5:131265852-131268218 | 53 | 173 | 81 | 85 | 27 | 33 | 85 | 28 |
| 5C_299_ENm002_FOR_7\|hg19\|chr5:131282811-131283081 | 76 | 76 | 35 | 12 | 22 | 16 | 30 | 17 |
| 5C_299_ENm002_FOR_8\|hg19\|chr5:131283081-131284754 | 22 | 292 | 94 | 58 | 75 | 73 | 59 | 16 |
| 5C_299_ENm002_FOR_9\|hg19\|chr5:131284754-131293292 | 20 | 497 | 32 | 21 | 157 | 83 | 195 | 110 |
| 5C_299_ENm002_FOR_10\|hg19\|chr5:131293292-131294942 | 81 | 226 | 103 | 99 | 57 | 19 | 35 | 12 |
| 5C_299_ENm002_FOR_11\|hg19\|chr5:131294942-131295972 | 57 | 169 | 17 | 46 | 56 | 13 | 26 | 0 |
| 5C_299_ENm002_FOR_12\|hg19\|chr5:131295972-131299135 | 52 | 467 | 114 | 221 | 122 | 48 | 27 | 57 |
| 5C_299_ENm002_FOR_13\|hg19\|chr5:131299135-131299252 | 71 | 265 | 32 | 16 | 43 | 35 | 61 | 19 |
| 5C_299_ENm002_FOR_15\|hg19\|chr5:131299620-131299809 | 6 | 134 | 30 | 3 | 14 | 16 | 25 | 1 |
| 5C_299_ENm002_FOR_16\|hg19\|chr5:131299809-131300294 | 65 | 415 | 24 | 53 | 33 | 28 | 45 | 41 |
| 5C_299_ENm002_FOR_17\|hg19\|chr5:131300294-131301697 | 76 | 1029 | 23 | 98 | 57 | 41 | 60 | 40 |
| 5C_299_ENm002_FOR_18\|hg19\|chr5:131301697-131308282 | 55 | 2606 | 146 | 36 | 81 | 51 | 146 | 36 |
| 5C_299_ENm002_FOR_20\|hg19\|chr5:131311855-131316841 | 7 | 149 | 27 | 3 | 11 | 0 | 2 | 0 |
| 5C_299_ENm002_FOR_21\|hg19\|chr5:131316841-131317443 | 73 | 307 | 100 | 72 | 62 | 28 | 34 | 9 |
| 5C_299_ENm002_FOR_22\|hg19\|chr5:131317443-131321565 | 26 | 1363 | 1482 | 669 | 250 | 105 | 171 | 42 |
| 5C_299_ENm002_FOR_24\|hg19\|chr5:131328527-131329090 | 62 | 238 | 2760 | 346 | 434 | 93 | 63 | 26 |
| 5C_299_ENm002_FOR_28\|hg19\|chr5:131336835-131337080 | 31 | 181 | 177 | 860 | 2251 | 77 | 76 | 40 |
| 5C_299_ENm002_FOR_29\|hg19\|chr5:131337080-131337292 | 6 | 91 | 16 | 265 | 1255 | 74 | 45 | 30 |
| 5C_299_ENm002_FOR_30\|hg19\|chr5:131337292-131338045 | 12 | 166 | 130 | 435 | 1635 | 124 | 74 | 10 |
| 5C_299_ENm002_FOR_31\|hg19\|chr5:131338045-131341758 | 78 | 297 | 68 | 280 | 434 | 129 | 94 | 49 |
| 5C_299_ENm002_FOR_32\|hg19\|chr5:131341758-131346229 | 28 | 393 | 365 | 100 | 248 | 1138 | 265 | 119 |
| 5C_299_ENm002_FOR_34\|hg19\|chr5:131348838-131356954 | 51 | 383 | 30 | 275 | 205 | 557 | 229 | 115 |
| 5C_299_ENm002_FOR_35\|hg19\|chr5:131356954-131363862 | 75 | 266 | 108 | 35 | 166 | 314 | 487 | 160 |
| 5C_299_ENm002_FOR_36\|hg19\|chr5:131363862-131363994 | 12 | 205 | 73 | 92 | 80 | 76 | 185 | 47 |
| 5C_299_ENm002_FOR_37\|hg19\|chr5:131363994-131368340 | 13 | 111 | 15 | 79 | 37 | 122 | 129 | 64 |
| 5C_299_ENm002_FOR_41\|hg19\|chr5:131384017-131385202 | 39 | 141 | 86 | 71 | 54 | 75 | 367 | 120 |
| 5C_299_ENm002_FOR_42\|hg19\|chr5:131385202-131391190 | 23 | 288 | 97 | 238 | 127 | 165 | 960 | 203 |
| 5C_299_ENm002_FOR_44\|hg19\|chr5:131405195-131408856 | 55 | 89 | 21 | 15 | 81 | 14 | 464 | 392 |
| 5C_299_ENm002_FOR_46\|hg19\|chr5:131414324-131416113 | 39 | 65 | 8 | 1 | 41 | 22 | 302 | 1528 |
| 5C_299_ENm002_FOR_47\|hg19\|chr5:131416113-131417265 | 3 | 49 | 7 | 3 | 63 | 6 | 156 | 313 |
| 5C_299_ENm002_FOR_48\|hg19\|chr5:131417265-131417742 | 16 | 47 | 11 | 12 | 37 | 41 | 187 | 524 |
| 5C_299_ENm002_FOR_49\|hg19\|chr5:131417742-131420182 | 3 | 21 | 32 | 6 | 47 | 55 | 408 | 319 |
| 5C_299_ENm002_FOR_51\|hg19\|chr5:131420579-131428367 | 1 | 91 | 12 | 24 | 27 | 14 | 256 | 236 |
| 5C_299_ENm002_FOR_52\|hg19\|chr5:131428367-131428520 | 3 | 47 | 15 | 6 | 22 | 12 | 262 | 135 |
| 5C_299_ENm002_FOR_53\|hg19\|chr5:131428520-131433879 | 58 | 126 | 87 | 72 | 95 | 33 | 435 | 300 |
| 5C_299_ENm002_FOR_56\|hg19\|chr5:131442826-131449474 | 37 | 108 | 7 | 35 | 30 | 54 | 468 | 201 |
| 5C_299_ENm002_FOR_57\|hg19\|chr5:131449474-131450295 | 2 | 134 | 44 | 11 | 41 | 66 | 196 | 192 |
| 5C_299_ENm002_FOR_61\|hg19\|chr5:131475645-131476657 | 7 | 10 | 9 | 4 | 14 | 14 | 130 | 42 |
| 5C_299_ENm002_FOR_62\|hg19\|chr5:131476657-131477534 | 11 | 21 | 11 | 2 | 15 | 12 | 109 | 87 |
| 5C_299_ENm002_FOR_63\|hg19\|chr5:131477534-131485214 | 52 | 175 | 86 | 15 | 41 | 40 | 487 | 246 |
| 5C_299_ENm002_FOR_64\|hg19\|chr5:131485214-131485878 | 21 | 108 | 15 | 12 | 23 | 41 | 255 | 89 |
| 5C_299_ENm002_FOR_68\|hg19\|chr5:131492484-131492991 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5C_299_ENm002_FOR_69\|hg19\|chr5:131492991-131494404 | 6 | 23 | 15 | 16 | 22 | 10 | 88 | 87 |
| 5C_299_ENm002_FOR_71\|hg19\|chr5:131495840-131515519 | 10 | 65 | 2 | 21 | 50 | 43 | 363 | 80 |
| 5C_299_ENm002_FOR_72\|hg19\|chr5:131515519-131516145 | 25 | 54 | 17 | 64 | 39 | 41 | 330 | 157 |
| 5C_299_ENm002_FOR_74\|hg19\|chr5:131526403-131528269 | 4 | 111 | 31 | 32 | 75 | 18 | 338 | 164 |
| 5C_299_ENm002_FOR_75\|hg19\|chr5:131528269-131530130 | 12 | 107 | 13 | 13 | 19 | 16 | 193 | 106 |

# Range of Values (K562)

Zoomed

# ChIA-PET Schematic

Fullwood et al,, Nature, 2009
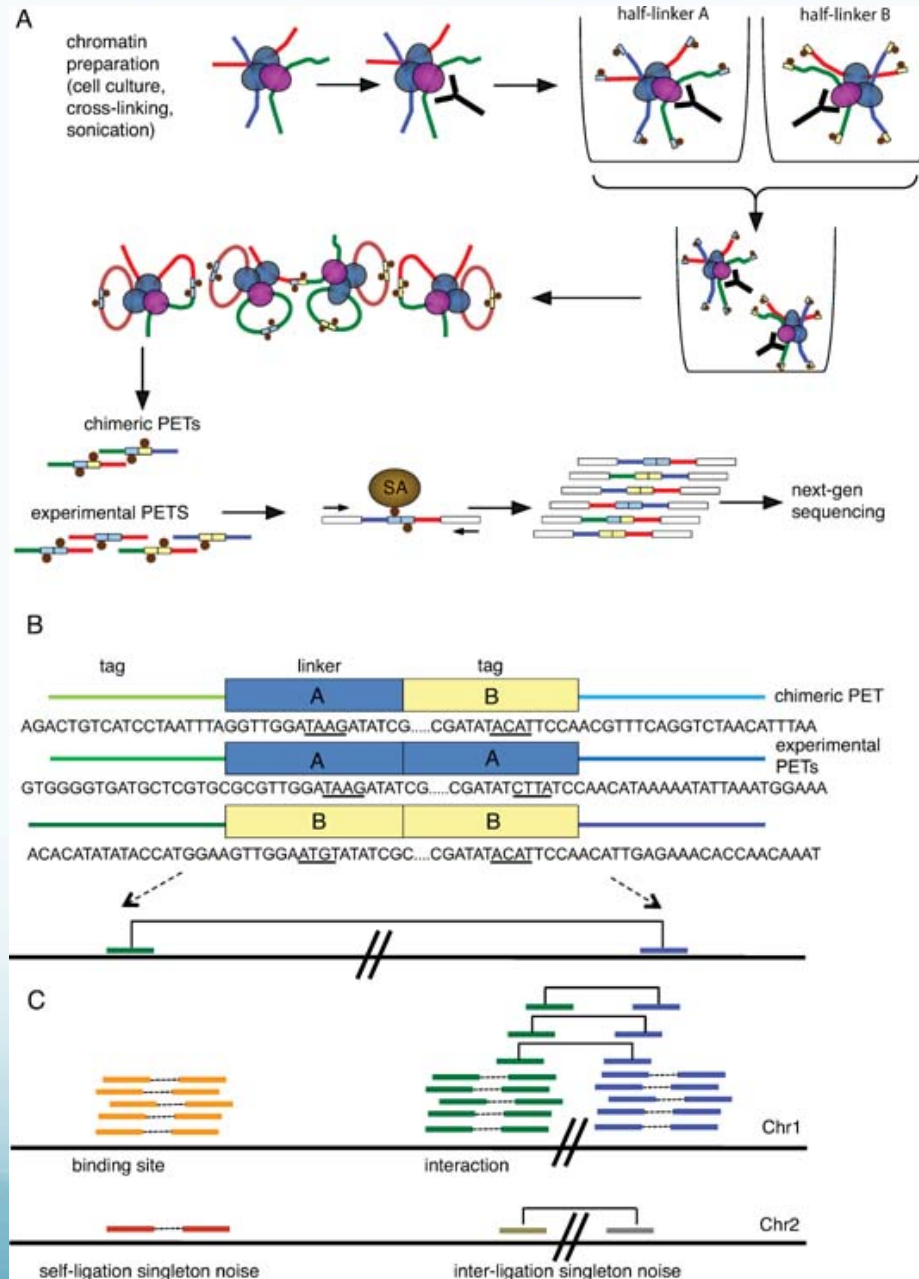
# Interaction Types



Complex

Cluster 1 — Cluster 2 — Cluster 3

Duplex

Cluster 1 — Cluster 2
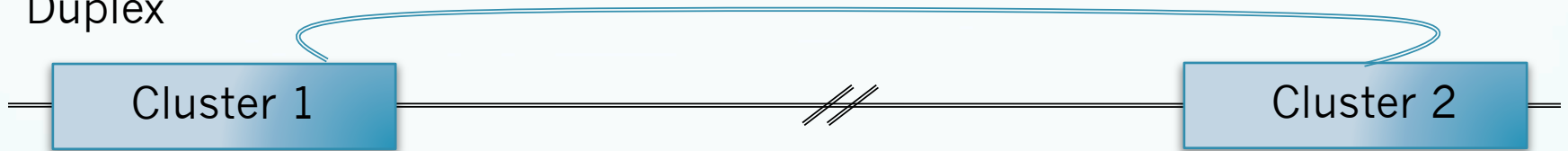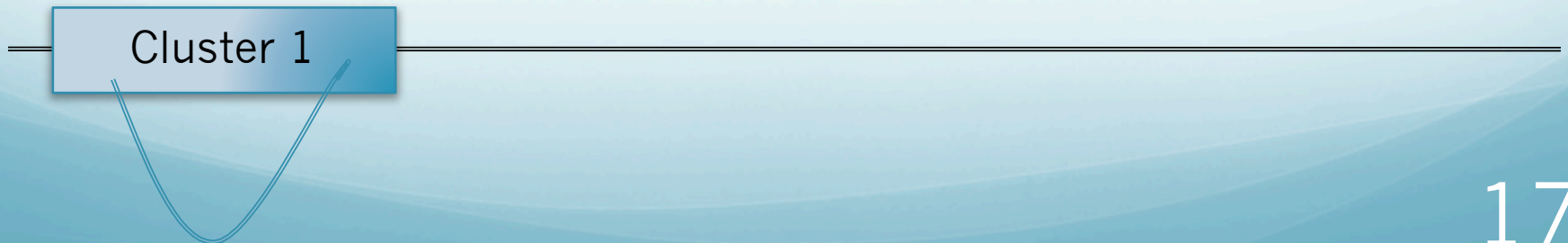
Self-ligation

Cluster 1
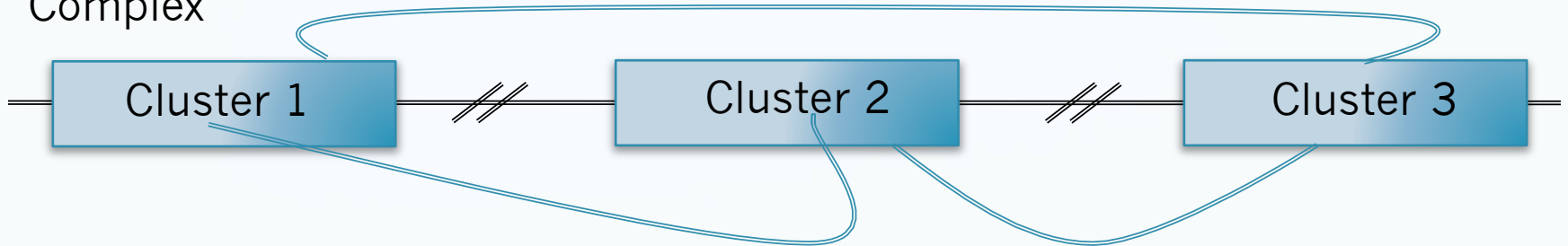
# ChIA-PET for ENCODE (so far)

- Pol II for five cancer lines and one normal line
  - HeLa (ovarian cancer)
  - MCF-7 (breast cancer)
  - HCT116 (colorectal cancer)
  - NB4 (acute promyelocytic leukemia)
  - K562 (acute myelogenous leukemia)
  - GM12878 (very recent development)


- Fullwood et al. (ER-alpha in MCF7)


- Ali Mortazavi's mouse experiments

# ChIA-PET Pilot Project (Pol II)

# Project Goals

- Emphasis of GIS
  - Characterize long-range interactions and define general transcriptional mechanisms

- My emphasis
  - Use ENCODE data to characterize the interacting regions and draw conclusions about various complexes that we can't discern from ChIP-Seq alone.

# Raw Sequencing Stats

**Table S1. RNAPII ChIA-PET library statistics summary**

| Library | Cell line | Total PET | Unique PET | Self-ligation PET | | Inter-ligation PET | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Intra-chromosome | | Inter-chromosome | |
| | | | | PET | Peak * | PET | Cluster † | PET | Cluster § |
| CHH524 | HCT116 | 226,903,653 | 8,749,703 | 3,126,311 | 17,394 | 363,493 | 1,719 | 5,209,961 | 17,545 |
| CHK004 | K562 | 153,421,905 | 23,188,484 | 3,983,892 | 13,112 | 1,198,730 | 1,990 | 17,869,121 | 15,696 |
| CHM040 | MCF7 | 129,114,582 | 38,356,322 | 5,920,613 | 10,370 | 2,329,229 | 5890 | 29,814,465 | 6,736 |
| CHM053 | MCF7 | 114,833,139 | 22,967,674 | 4,817,946 | 11,234 | 1,380,036 | 4,146 | 16,470,458 | 3,965 |
| IHH025 | HeLa | 193,471,470 | 19,079,666 | 1,718,224 | 15,333 | 1,015,674 | 13,833 | 16,267,788 | 187,119 |
| IHN009 | NB4 | 138,257,582 | 14,023,893 | 876,773 | 12,707 | 722,957 | 3,711 | 12,388,855 | 50,521 |
| IHN010 | NB4 | 127,974,844 | 15,198,626 | 1,166,267 | 13,828 | 786,771 | 4,773 | 13,200,381 | 61,578 |
| RNAPII | MCF7 | 243,947,721 | 61,319,903 | 10,734,769 | 11,159 | 3,709,079 | 14,523 | 46,284,757 | 17,173 |
| RNAPII | NB4 | 266,232,426 | 29,173,744 | 2,031,512 | 13,890 | 1,507,342 | 13,595 | 25,554,804 | 189,678 |
| H-RNAPII | All | 969,144,036 | 118,523,881 | 16,756,994 | 14,604 | 6,415,122 | 67,746 | 94,715,066 | 825,245 |

Note: *Overlap PET count 5+, FDR <0.05; †Overlap PET count 2+, FDR<0.05, genomic distance > 8Kb; §Overlap PET count 2+, FDR <0.05

# Using ChIA-PET to Explore Transcription and Nuclear Organization

# General Profile and Signal Characteristics
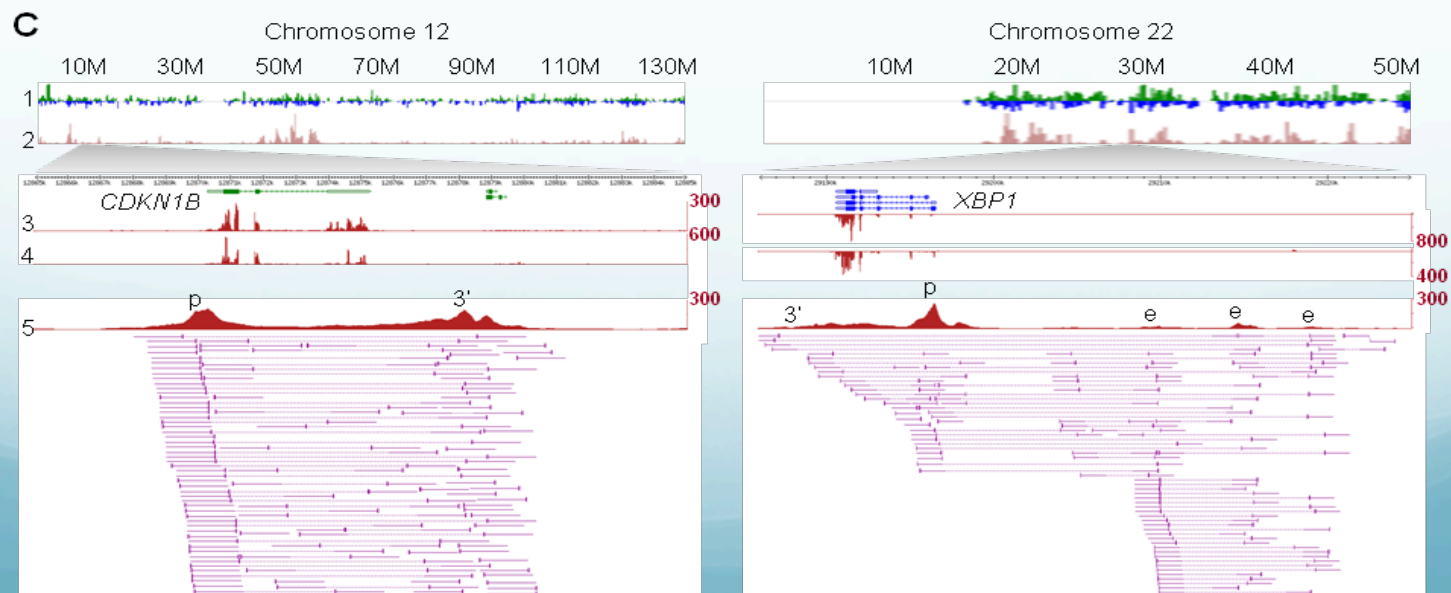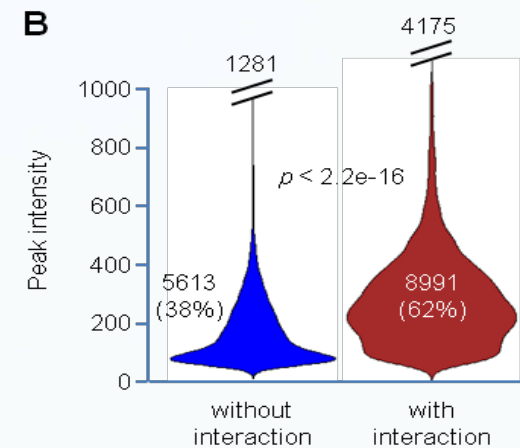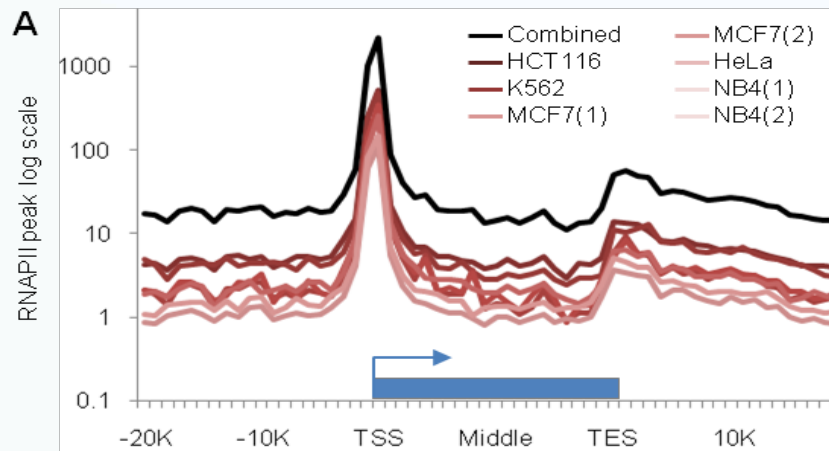
# Example of Domain Segmentation
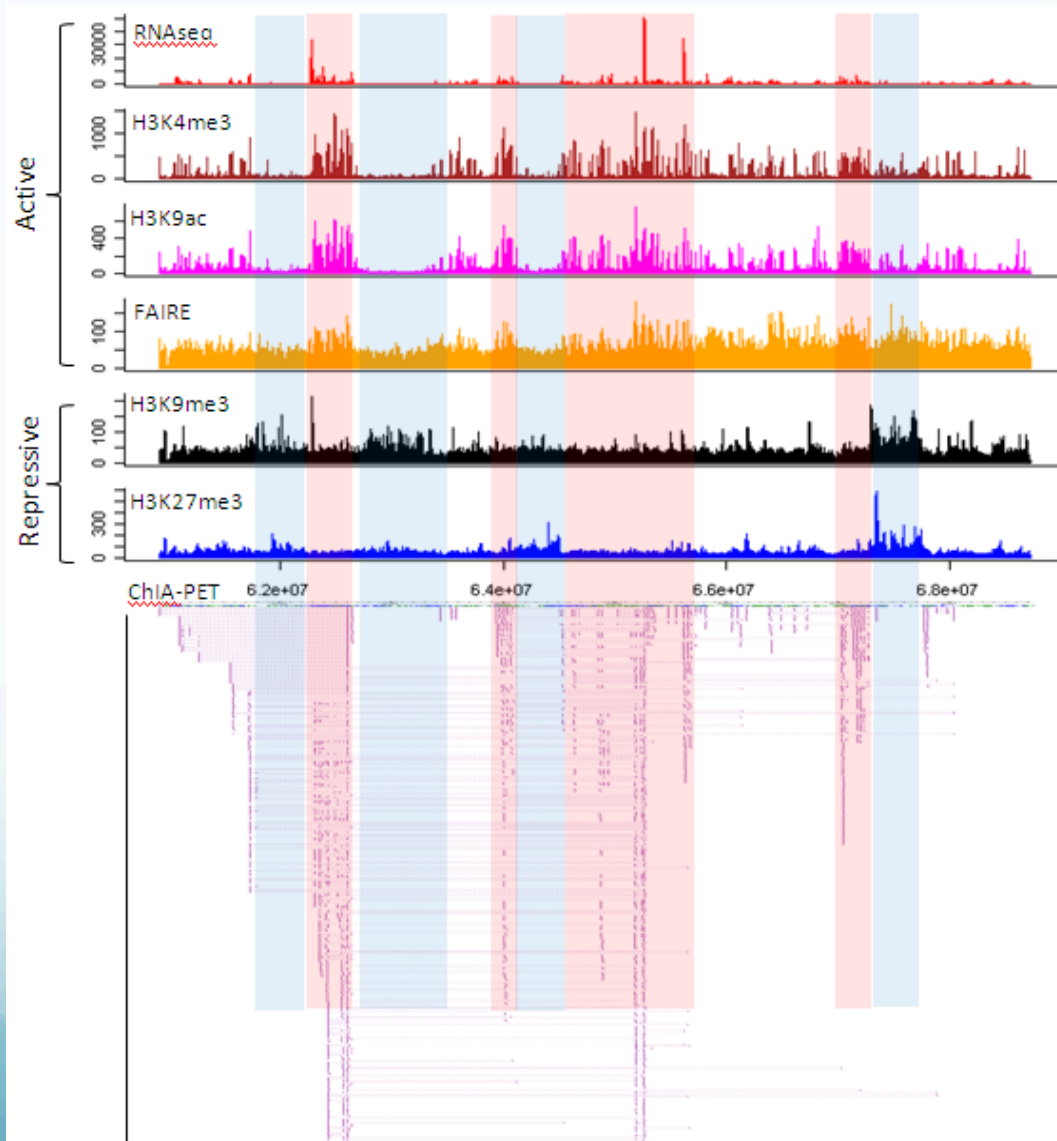
# Chromatin models for transcription regulation



**A** Basal Promoter Model
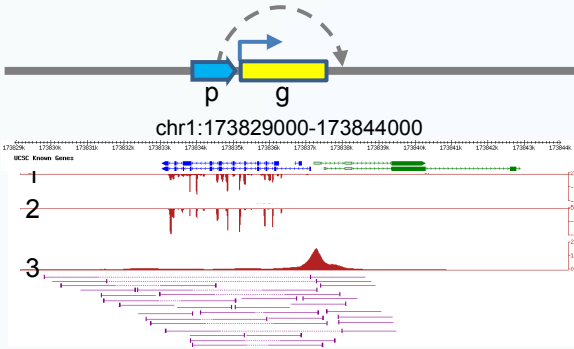
**B** Enhancer Model

**C** Chroperon Model

**D**

**E**
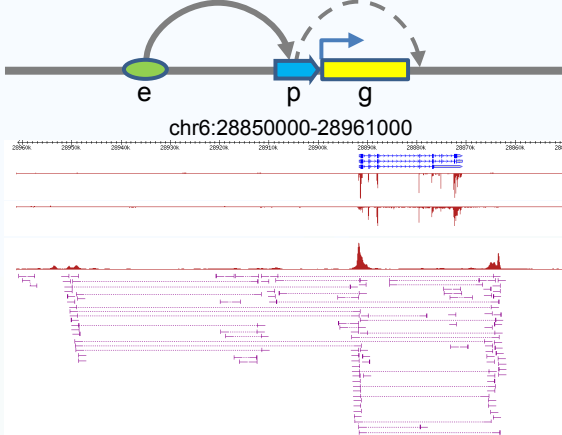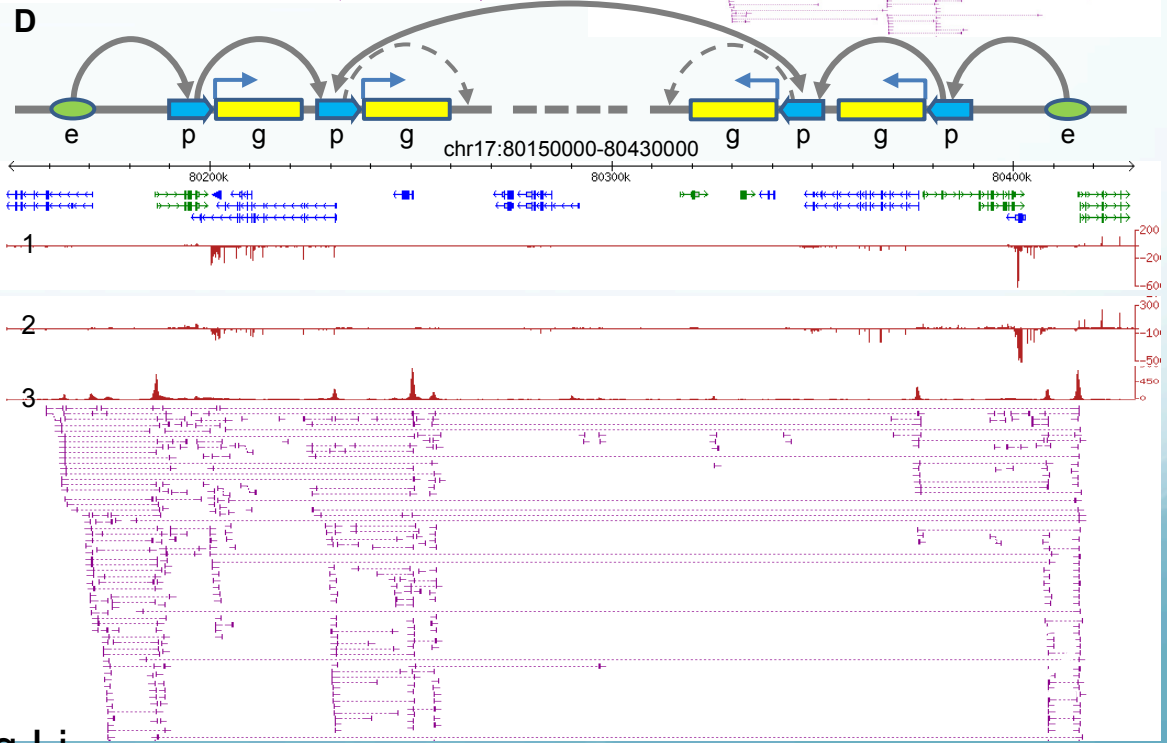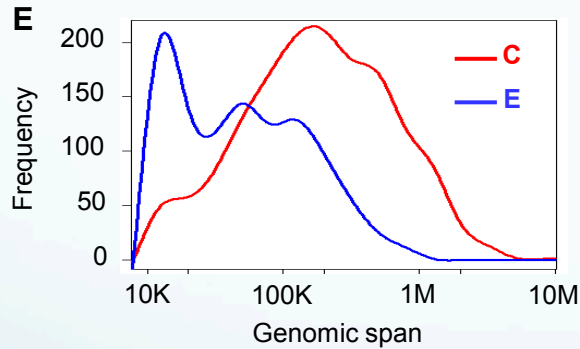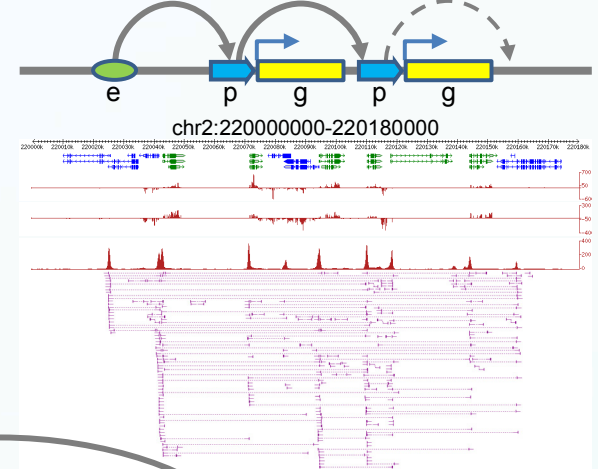
**F** Number of chromatin structures
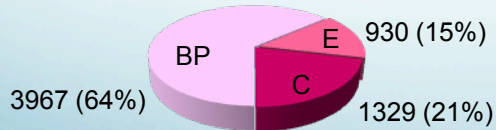
Number of genes involved
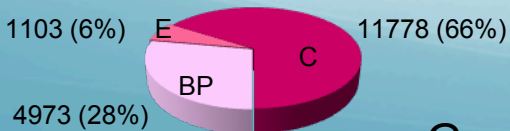
# Epigenomic Features of Chromatin Interaction Models

Epigenomic features of chromatin interaction models
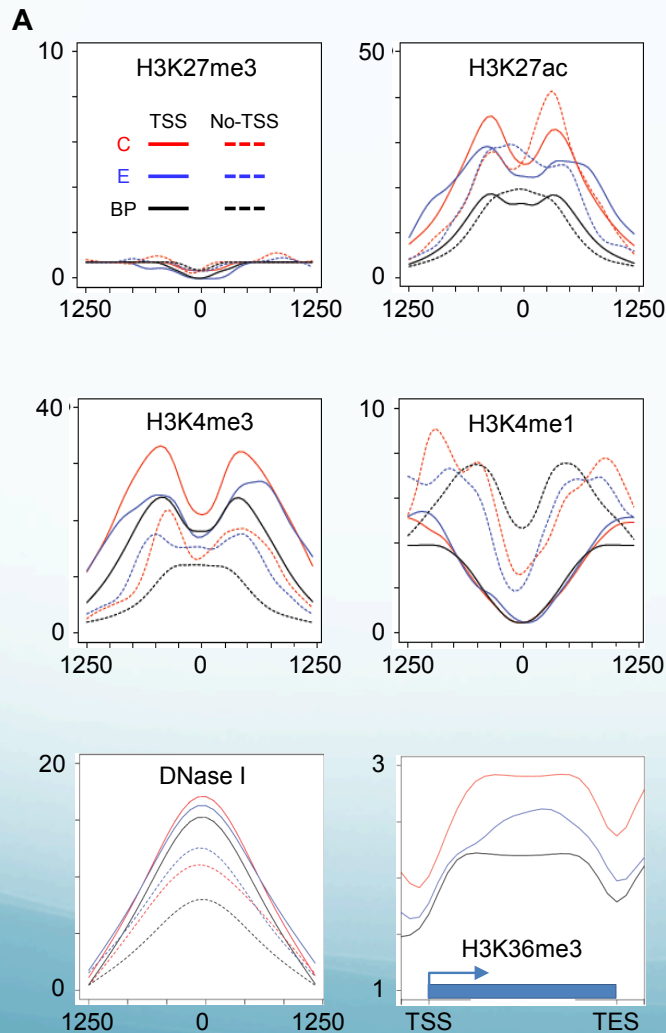
# RNAPII and Gene Expression
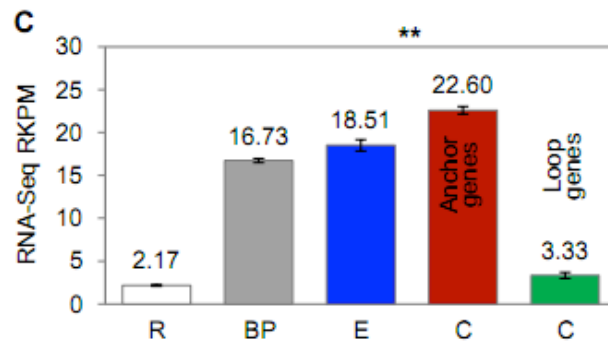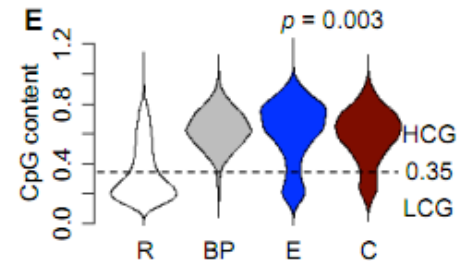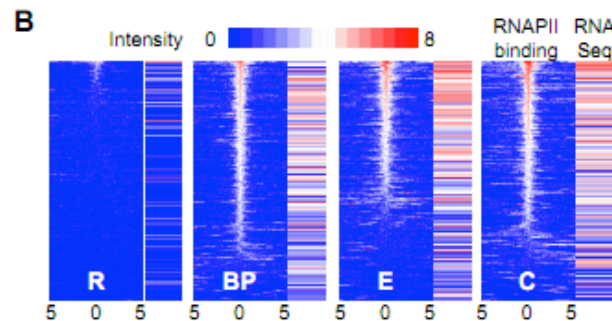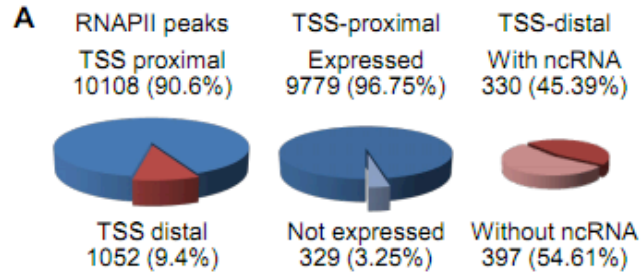
# ChIA-PET and TF Complexes

# Targeted Recruitment



Isolated by ChIP
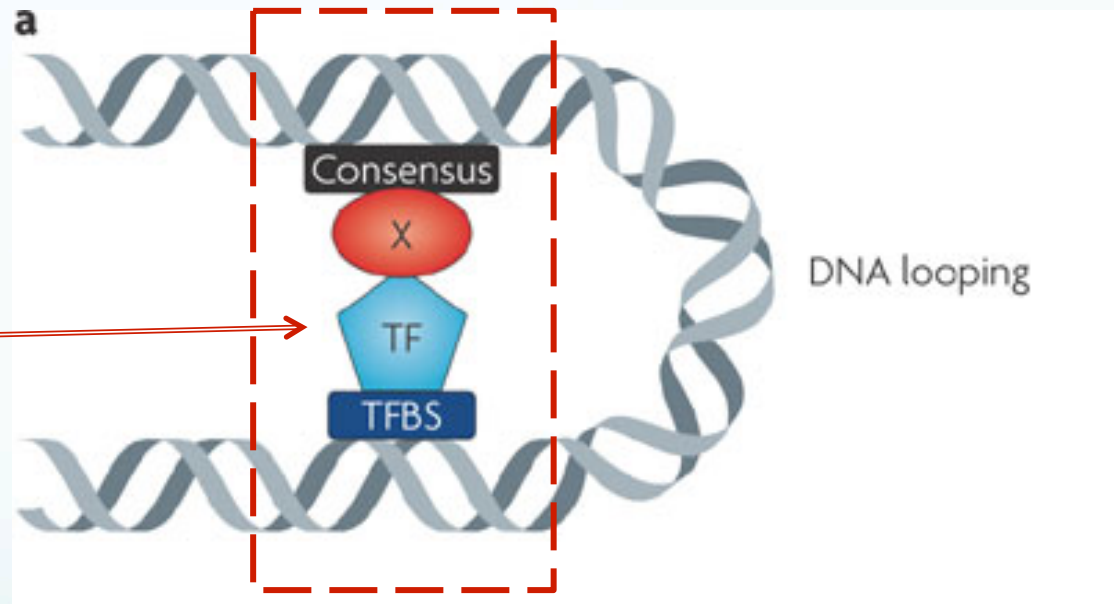
PJ Farnham, Nature Reviews Genetics, 2009

# ChIA-PET Anchors and TF Complexes in Chroperons (K562)



Pairwise Interactions of TFs at Operon Model Anchor Sites
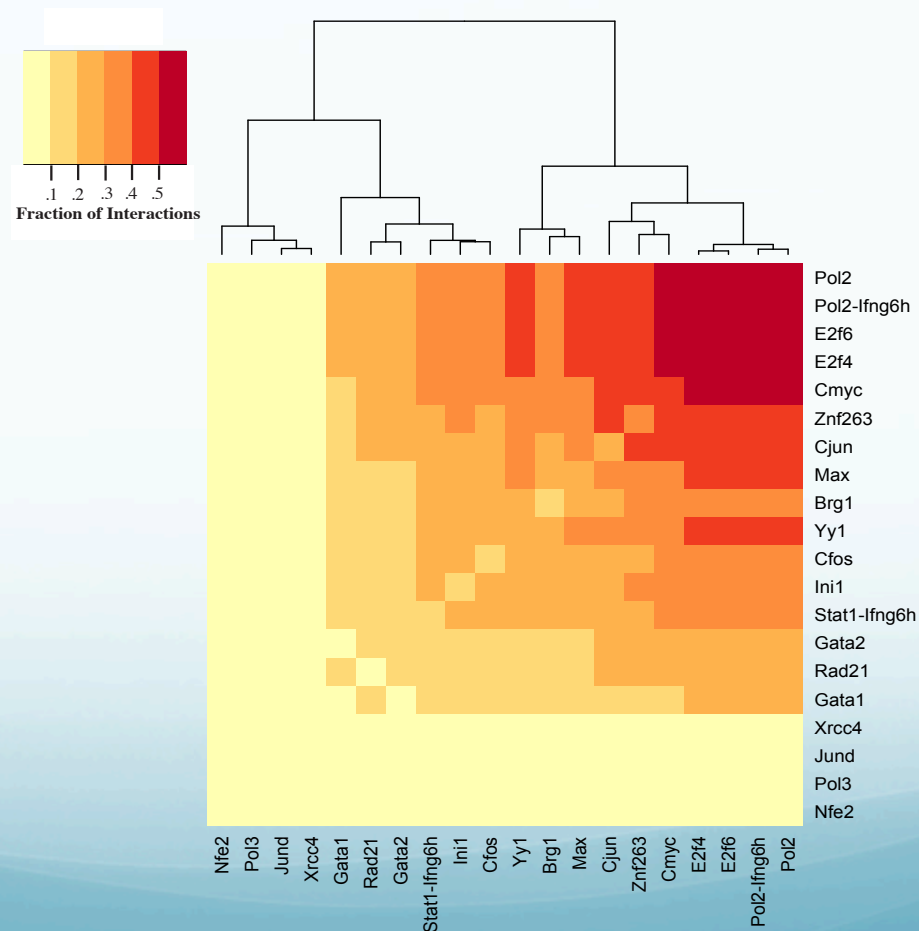
# Promoter-associated TFs

# Distal TFs

### Cfos



### Cjun



### Gata1



### Gata2



### Stat1



### Stat2



### JunD



### Max
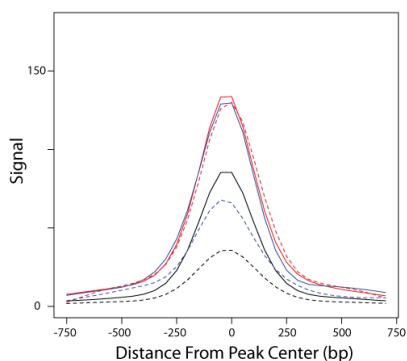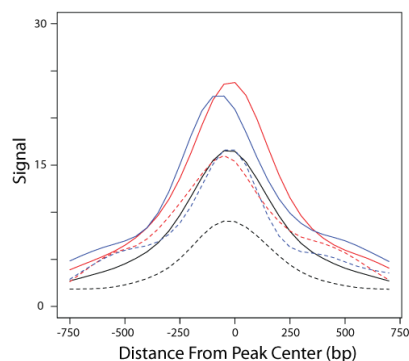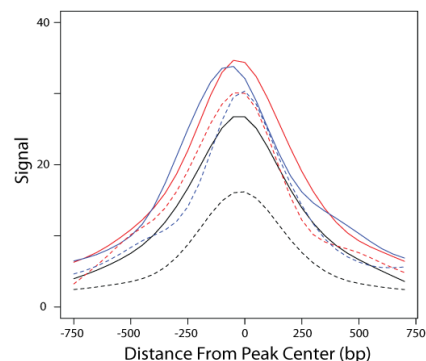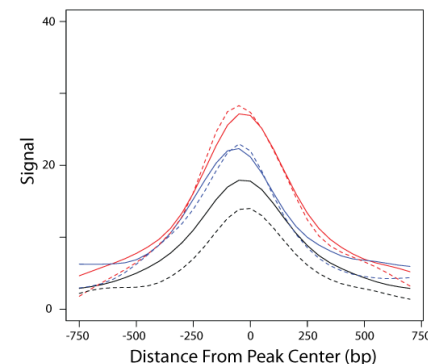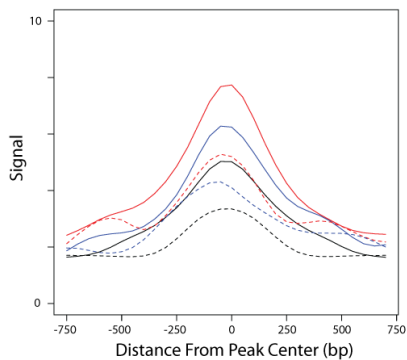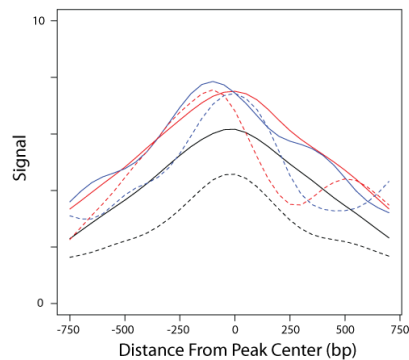


### Nfe2



Chroperon    Enhancer-Promoter    No Interaction    TSS-associated    Not TSS-associated
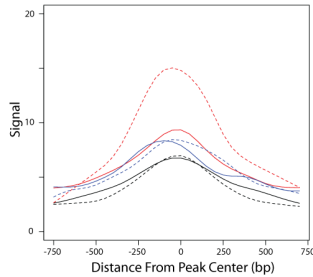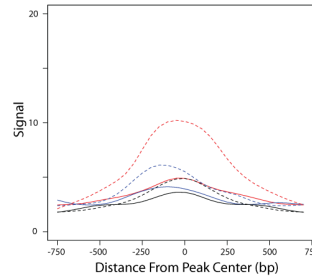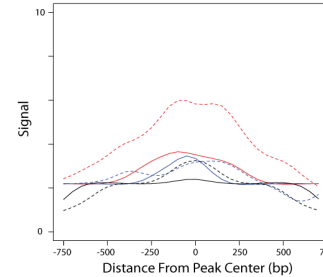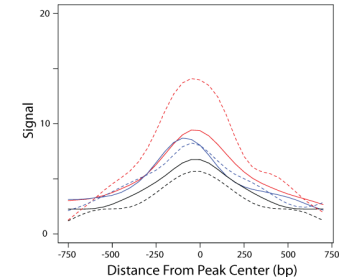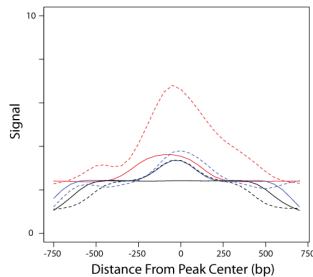
# Interesting Factors



Rad21  Ini1  Brg1  CTCF

Legend: Chroperon (red), Enhancer-Promoter (blue), No Interaction (black), TSS-associated (solid), Not TSS-associated (dashed)

# Conclusions

- Chroperon Structure
  - Expression patterns and histone modification profiles

- A better sense of which complexes arise due to looping
  - Also which components are proximal vs. distal

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# Switching Gears



No, it isn't (quite) Mt. Everest...
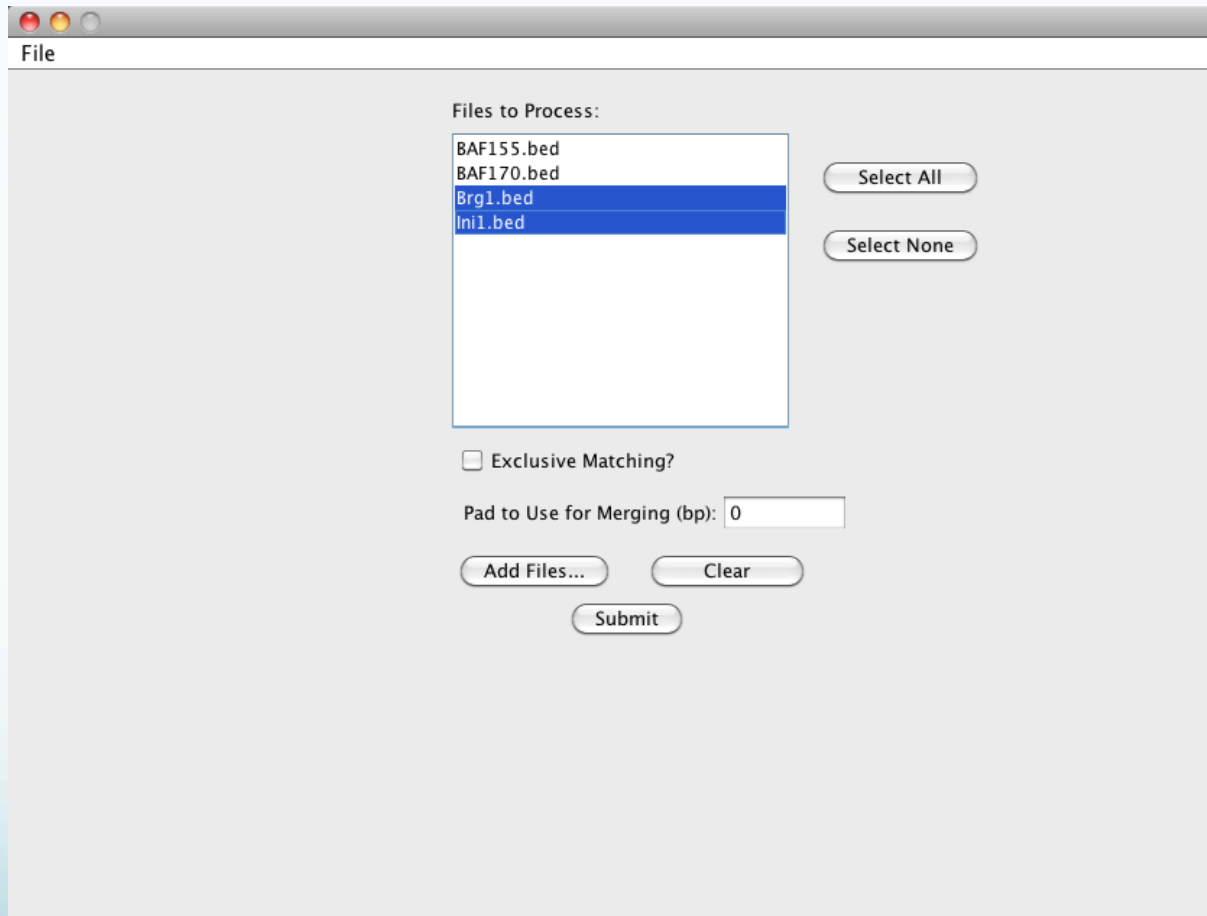
# Project Goals

- Design a set of tools to assist the average user with analyzing ChIP-Seq data
    - Tool to identify putative TF complexes
        - BEDTools does a similar task, but less user friendly for a biologist
    - Other tools being discussed with Arif and Joel
        - Tool or method to relate shapes of peaks
        - Tool to combine multiple signal tracks into a single track

# Tool to Identify TF Complexes

- Designed for ease of use
  - Java Web Start, portable, GUI
  - Link: http://homes.gersteinlab.org/people/rka24/BIMT/launch.html
  - Manual: http://homes.gersteinlab.org/people/rka24/BIMT/BIMT_Manual.doc

# Input

# Graphical Results

# Text Results

# Future Plans

- Finish revisions for ChIA-PET manuscript and analyses

- Creation of a computational tool or tool set relating to ChIP-Seq data

- Interview

- Give another group meeting

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