Genome-wide flame feature detection pipeline for Hi-C chromatin conformation maps

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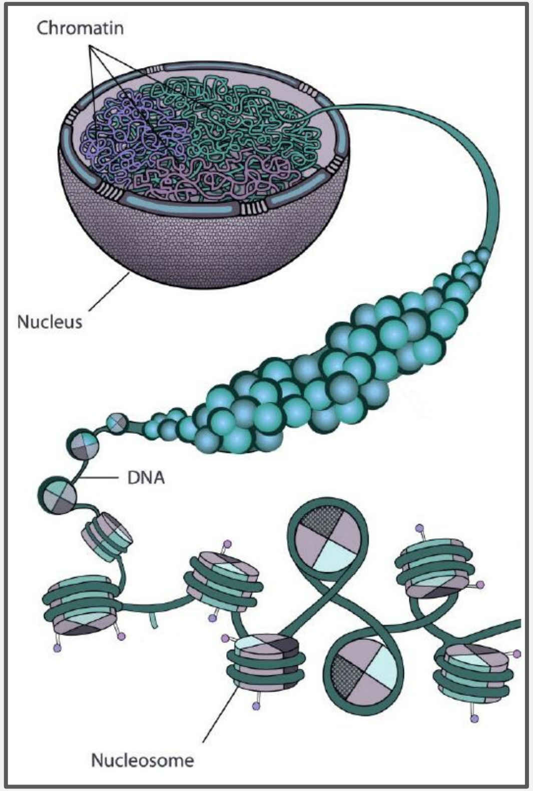
Mirny Lab

Chromatin

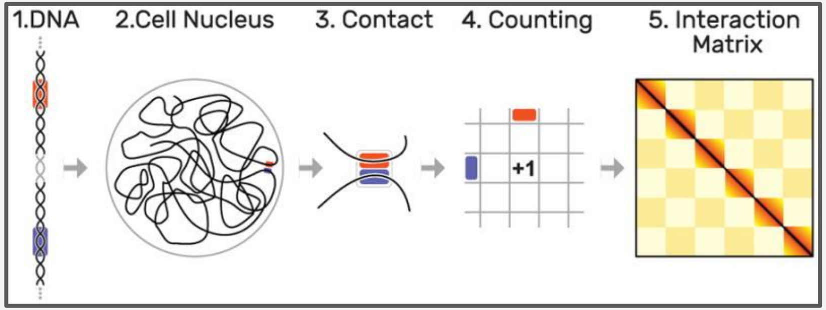
- Complex of DNA coiled around histone proteins
- Efficiently packages 2 m genome into 4-8 µm nucleus
- Preserves structure and sequence



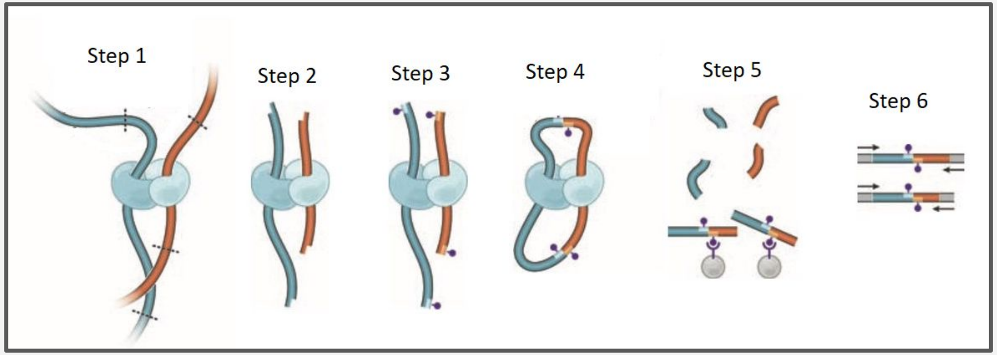
Background

Hi-C chromatin conformation heatmaps

- Genome-wide interaction maps
- Darker index indicates higher interaction between those two genomic loci
- Computed over ensemble average of over 10^7 cells
- Symmetric about the diagonal
- Checkerboarding pattern due to phase separation



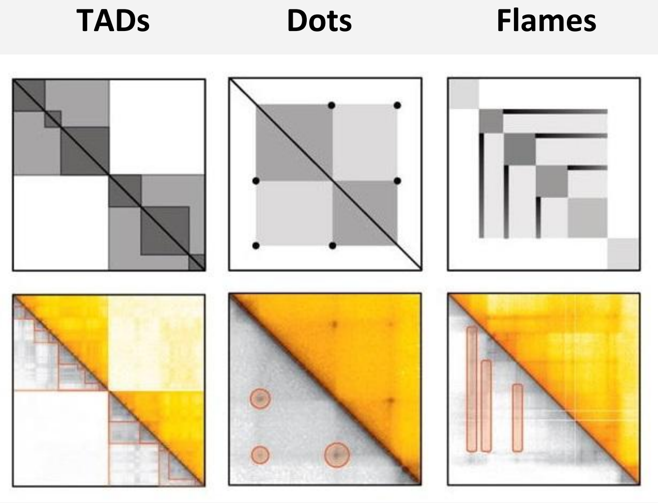
Gathering Hi-C data



1. Crosslink DNA
2. Cut with restriction enzyme
3. Fill and mark ends with biotin
4. Re-ligate
5. Purify and Sheer DNA
6. Sequence using paired-ends

Hi-C visual features

Definitions



Topologically Associating Domains (TADs)

- contiguous regions of increased contact frequency
- appear as relatively insulated squares

Dots

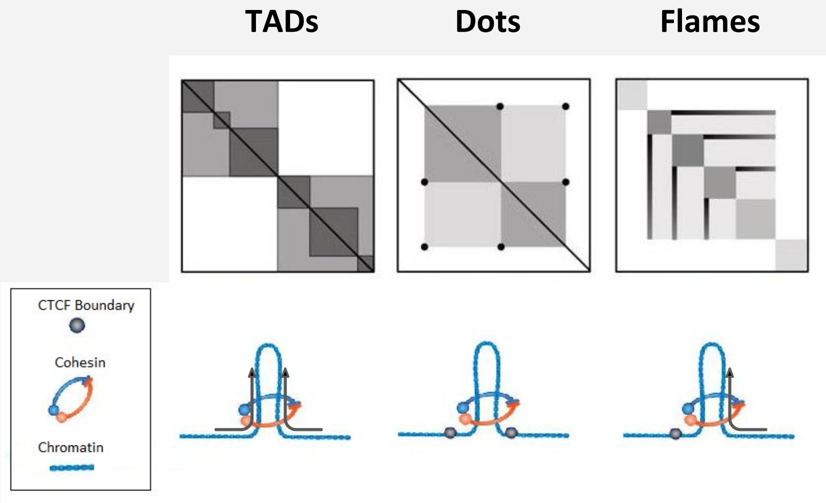
- Small circular regions of increased contact frequency
- Appear on corners of TADs

Flames

- Horizontal or Vertical linear regions of increased contact frequency
- Occasionally appear on border of TADs

Hi-C visual features

Loop Extrusion Mechanisms



Topologically Associating Domains (TADs)

- Cohesin is not blocked by CTCF on either side of chromatin fiber
- Loop is extruding through both sides of chromatin fiber

Dots

- Cohesin is blocked by CTCF on both sides of chromatin fiber
- Loop is temporarily immobile

Flames

- Cohesin is blocked by CTCF on one side of chromatin fiber
- Loop is extruding through one side of chromatin fiber

Research Question

How can we computationally locate and demarcate all flames within a Hi-C map?

Observations

- Classic line detection algorithms look for high variation between adjacent pixels
- Hi-C maps contain too much noise for classic line detection algorithms

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Custom image processing pipeline

Methods

Observed Over Expected Normalization

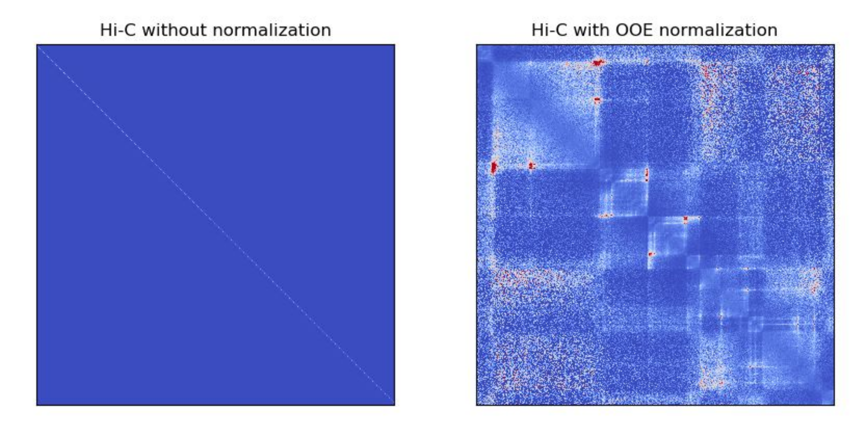
Step 1

Motivation

- Visual features are lost because relative intensity of main diagonal is so strong
- Need to counteract distance decay along main diagonal

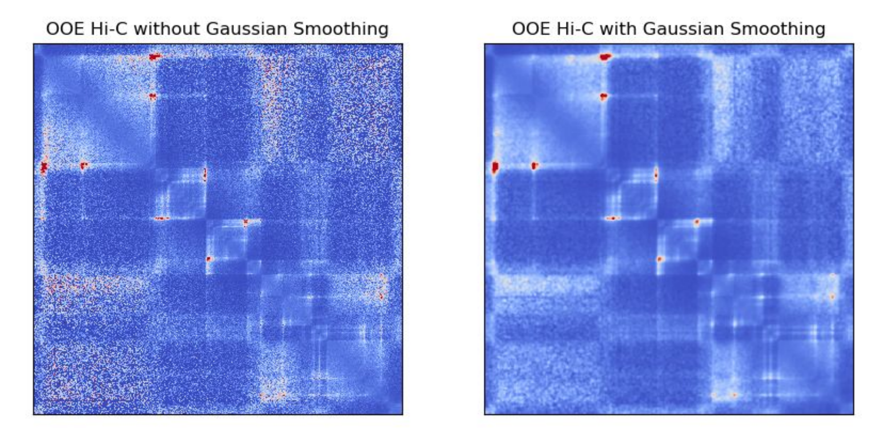
Mechanism

- Compute mean along each adjacent diagonal
- Divide values along diagonal by computed mean



Gaussian Smoothing

Step 2



Motivation

- Need to reduce noise to accentuate boundaries of flames
- Apply Gaussian filter (a type of low-pass filter) to smooth over entire map, reducing noise
- Preserves edges better than mean filter

Mechanism

- Normally distributed kernel computed by:

$$\boxed{G(x,y) = \frac{1}{2\pi\sigma^2} e^{-\frac{x^2+y^2}{2\sigma^2}}}$$

  - x is the x-coordinate
  - y is the y-coordinate
  - σ is the standard deviation of the distribution (optimized at 1.5)

- Kernel convolves around Hi-C map
- Outputs “weighted average” of each pixel’s neighborhood, average weighted towards central pixels

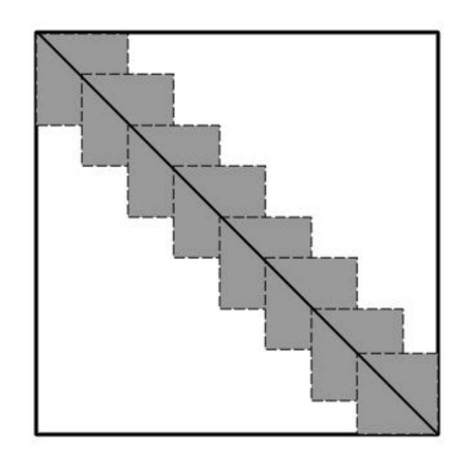
Diagonal Slicing

Step 3

Motivation

- The scale of a single Hi-C maps is measured in megabases (1,000,000 bases), which is too large
- One pass through the entire genome fails to pick up smaller features
- Looking at sub-regions in close proximity to main diagonal increases efficiency and accuracy

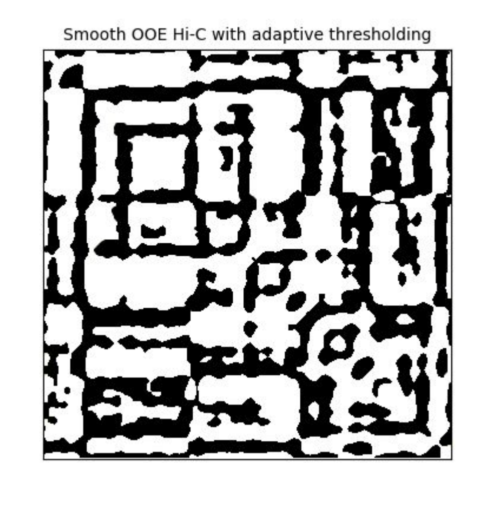
Sub-regions indicated by gray squares



Methods

Adaptive Thresholding

Step 4



Motivation

- Need to separate visual features from surrounding data in the Hi-C Map
- Accomplished by binarization with respect to a threshold

Mechanism

- Computes binary thresholded mask image based on local pixel neighborhood
- Threshold value:
    - Weighted mean for local pixel neighborhood of radius 20
    - Subtracted by a constant (optimized to 0.01)

Methods

Skeletonization

Step 5

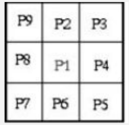
Motivation

- Reduce binary Hi-C to its structural skeleton to isolate flames

Mechanism

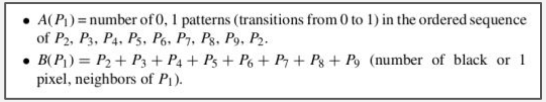
- Applying Zhang-Suen Thinning Algorithm to binary Hi-C map:

8 pixel local neighborhood:



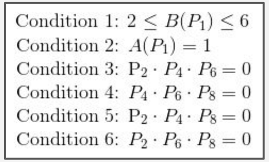
P9	P2	P3
P8	P1	P4
P7	P6	P5

Equations:

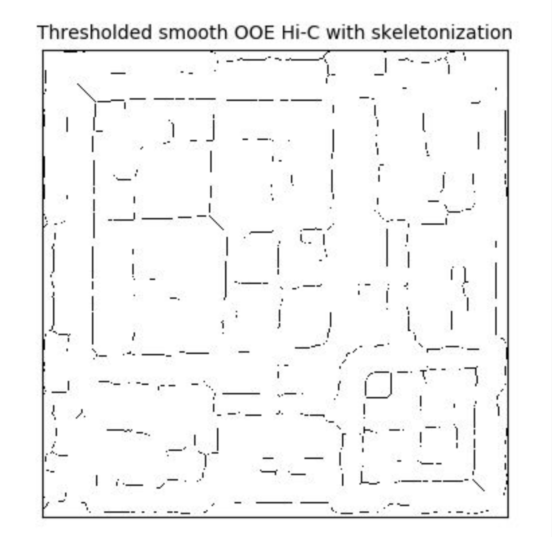


- $A(P_1)$ = number of 0, 1 patterns (transitions from 0 to 1) in the ordered sequence of $P_2, P_3, P_4, P_5, P_6, P_7, P_8, P_9, P_2$.
- $B(P_1) = P_2 + P_3 + P_4 + P_5 + P_6 + P_7 + P_8 + P_9$ (number of black or 1 pixel, neighbors of P_1).

Conditions to turn P_1 from black to white:



Condition 1: $2 \leq B(P_1) \leq 6$
Condition 2: $A(P_1) = 1$
Condition 3: $\mathrm{P}_2 \cdot P_4 \cdot P_6 = 0$
Condition 4: $P_4 \cdot P_6 \cdot P_8 = 0$
Condition 5: $\mathrm{P}_2 \cdot P_4 \cdot P_8 = 0$
Condition 6: $P_2 \cdot P_6 \cdot P_8 = 0$



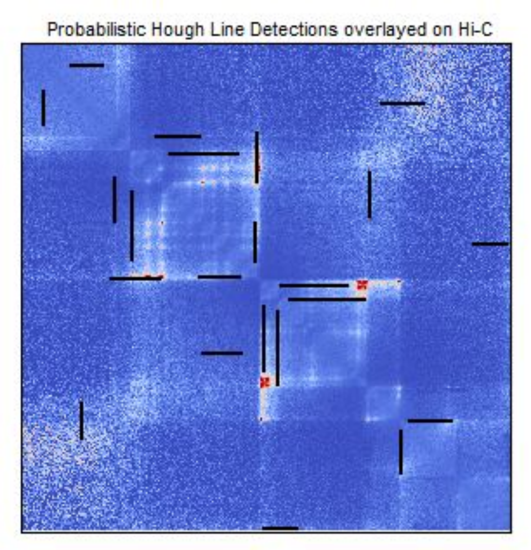
Thresholded smooth OOE Hi-C with skeletonization

Probabilistic Hough Transform

Step 6

Motivation

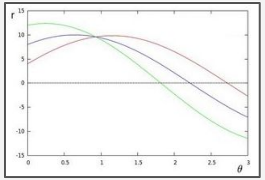
- Need algorithm to demarcate flames from skeleton binary Hi-C
- Should be computationally efficient given size of Hi-c maps



Probabilistic Hough Line Detections overlayed on Hi-C

Mechanism

- Standard Hough Transform:
  - Represent line in polar form: $\boxed{r = x\cos\theta + y\sin\theta}$
  - Define family of lines going through point (x_0, y_0): $\boxed{r_\theta = x_0 \cdot \cos\theta + y_0 \cdot \sin\theta}$
  - Example: Family of lines going through points (8, 6), (4, 9), (12, 3) in Θ-r plane:



  - Line containing all three points defined by intersection r = 9.6, Θ = 0.925
  - Hough Transform tracks intersections between curves of every point using discrete accumulator matrix
  - If number of intersections greater than predefined threshold, line is detected with (Θ, r) at intersection point
- Probabilistic Hough Transform:
  - Uses randomly selected subset of all points in image for increased efficiency

Line Length Thresholding

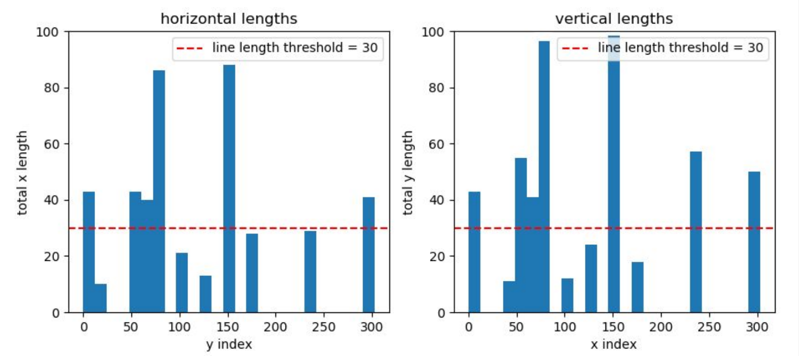
Step 7

Motivation

- Need method to reduce false positives among detected lines
- Along any horizontal or vertical loci, the larger the total length of detected lines, the higher the probability of flame existence

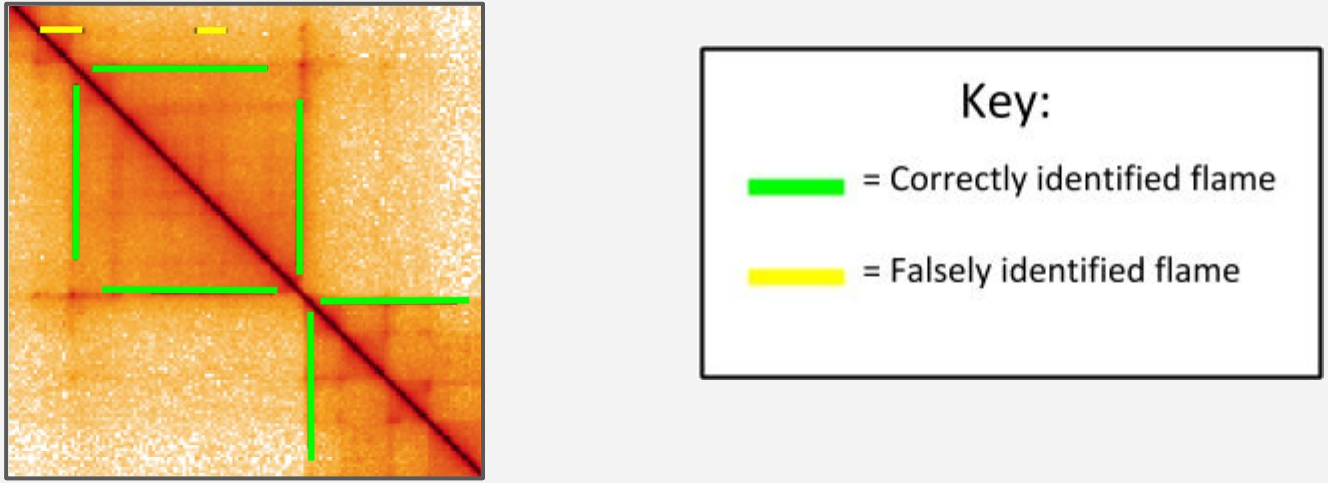
Mechanism

- Compute total detected line length along all horizontal and vertical loci
- Thresholded at length of 30, according to histogram
- Any horizontal or vertical loci with total detected line length greater than 30 considered a flame



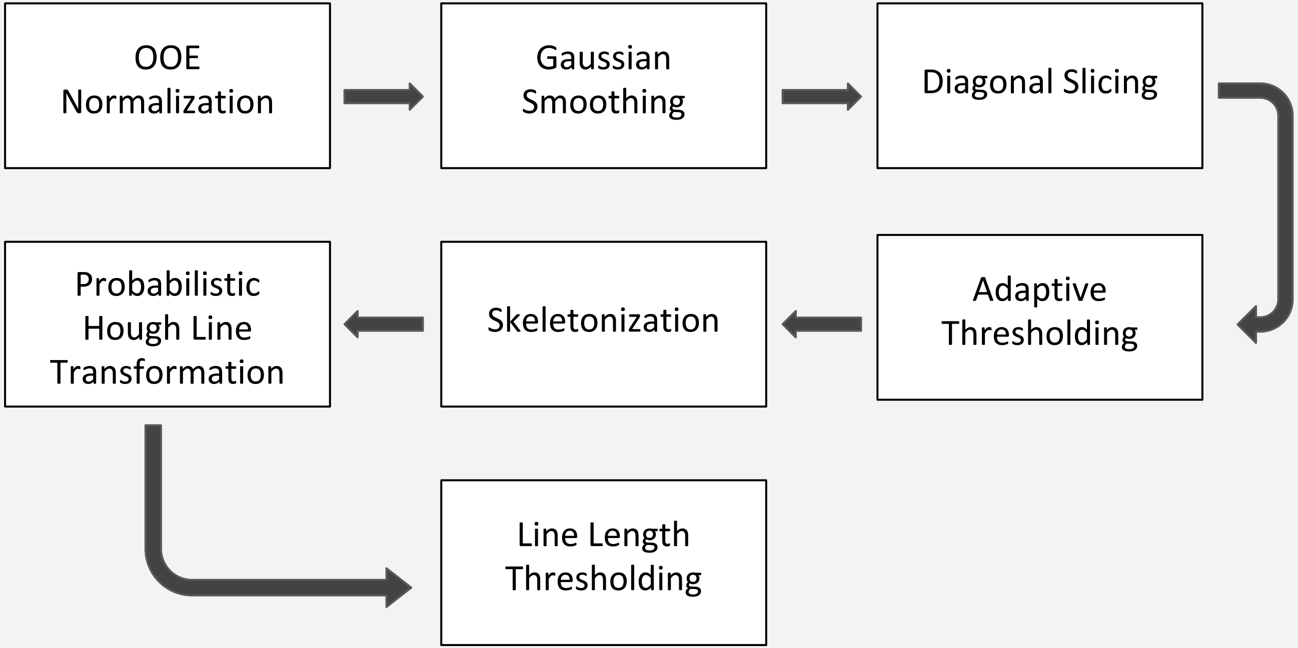
Genome-wide flame demarcation on HiGlass viewing platform

Sample taken from HiGlass:



Conclusion

We created a complete image processing pipeline to delineate flames within Hi-C maps:



Future Research

1. Reduce false positives
   - Further optimize parameters across entire pipeline

2. Biological implications of flames
   - Analysis of flames on a larger scale is now possible

References

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Acknowledgments

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Questions?