Topologically associating domains of chromatin: methods and tools for calling

Part 1

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Group meeting at BI
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1. Introduction

2. Topologically associating domains
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**Question:** How is chromatin folded within euchromatin and heterochromatin compartments?
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The answer came with the development of chromatin conformation capture methods (3C, 2002; 4C, 2006; 5C, 2006; Hi-C, 2009).
Hi-C experiment scheme:

- Crosslink DNA
- Cut with restriction enzyme
- Fill ends and mark with biotin
- Ligate
- Purify and shear DNA; pull down biotin
- Sequence using paired-ends

Lieberman-Aiden et al., 2009
Introduction

Chromosome is split into \( r \) bp bins (\( r \) is called contact matrix resolution).

**Contact matrix** \( C \) is built: \( C(i, j) \equiv C(j, i) \) is a number of paired-end reads such that one read was mapped into bin \( i \) and the other read was mapped into bin \( j \). Contact matrix is usually represented as a heatmap.
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Self-interacting domains can be seen on the main diagonal of a contact matrix (Dekker et al., 2013, adapted).
Dixon et al., 2012 found self-interacting domains in human and mouse using Hi-C data.

LETTER

doi:10.1038/nature11082

Topological domains in mammalian genomes identified by analysis of chromatin interactions

Jesse R. Dixon¹ ² ³, Siddarth Selvaraj¹ ⁴, Feng Yue¹, Audrey Kim¹, Yan Li¹, Yin Shen¹, Ming Hu⁵, Jun S. Liu⁵ & Bing Ren¹ ⁶
Dixon et al., 2012 found self-interacting domains in human and mouse using Hi-C data. They called such domains **topologically associating domains (TADs)**. TAD is such a region that frequency of intra-TAD interactions is higher than inter-TAD interactions.
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- Similar domains were found in *Drosophila* genome in the same year: Sexton et al., 2012; Hou et al., 2012.
- TADs were also found in the same year in mouse X chromosome by Nora et al., 2012.
Topologically associating domains

Nguyen H. G. and Bosco G., 2015

- TADs are collections of many chromatin loops.
- TADs are separated by TAD borders (intervening chromatin).
- Mammalian TAD borders are enriched in active transcription, housekeeping genes, tRNA genes and SINE repeats, as well as binding sites for the architectural proteins CTCF and cohesin (Dekker J. and Heard E., 2015).
TAD-like domains were found in several organisms in 2012 – 2015 (Dekker J. and Heard E., 2015, adapted).
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- TADs have hierarchical folding and consist of sub-TADs (Cubeñas-Potts C. and Corces V. G., 2015; Rao et al., 2014).
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Self-interacting domains in other organisms can have different functions (Dekker J. and Heard E., 2015).
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Let’s partition each chromosome into $r$ bp bins, where $r$ is a contact matrix resolution.

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Then $A_L(i)$ is a number of read pairs that map from the bin $i$ to the upstream $L$ bp.

And $B_L(i)$ is a number of read pairs that map from the bin $i$ to the downstream $L$ bp.
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\[ A_L(i) = 7 \quad \text{and} \quad B_L(i) = 2 \]
At the end of a TAD we expect a bias in contact frequency towards upstream regions.

And vice versa: at the beginning of a TAD we expect a bias in contact frequency towards downstream regions.
We can use this bias for TAD calling. Consider some bin $i$ and its $L$ bp vicinity. Let $A \equiv A_L(i)$, $B \equiv B_L(i)$, $D \equiv D_L(i)$, and $E \equiv E_L(i)$. Then, let's define **directionality index** (Dixon et al., 2012)

$$DI = \frac{B - A}{|B - A|} \left(\frac{(A - E)^2}{E} + \frac{(B - E)^2}{E}\right),$$

where $E \equiv E_L(i) = \frac{A_L(i) + B_L(i)}{2}$ is an expected number of reads (without the upstream or downstream contact frequency bias).
We can use this bias for TAD calling. Consider some bin \( i \) and its \( L \) bp vicinity. Let \( A \equiv A_L(i) \), \( B \equiv B_L(i) \), \( D \equiv D_L(i) \), and \( E \equiv E_L(i) \). Then, let’s define **directionality index** (Dixon et al., 2012)

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At the end of a TAD DI should have a local minimum, and immediately at the beginning of the next TAD DI should have a local maximum.
An illustration of this idea from Dixon et al., 2012 (Hi-C data for hESC – human embryonic stem cell line, some region of chr2):
DI calculation from a contact matrix (fig. is based on Crane et al., 2015):
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DI = \frac{\sum B - \sum A}{|\sum B - \sum A|} \left( \frac{(\sum A - E)^2}{E} + \frac{(\sum B - E)^2}{E} \right),
\]

where \( E = \frac{\sum A + \sum B}{2} \), \( \sum A \) and \( \sum B \) are sums of elements in contact submatrices A and B, respectively.
Now we can define a Hidden Markov Model (HMM) for TAD calling with DI (Dixon et al., 2012):
Baum-Welch algorithm was used (somehow...) to compute maximum likelihood estimates of the model and the parameter estimates of transition and emission.

Forward-backward algorithm was used to estimate posterior marginals, i.e., $P(q_t = q | D_1 = d_1, D_2 = d_2, \ldots, D_n = d_n)$, where $q$ is a hidden state, $t \in \{1, \ldots, n\}$, $d_1, d_2, \ldots, d_n$ are emission values.

For each chromosome the authors tried to use 1–20 mixtures of Gaussians and chose one set with the best goodness of fit using the AIC criterion: $AIC = 2k - 2 \ln(L)$, where $k$ is the number of parameters in the model and $L$ is the maximum likelihood estimate.
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**TAD borders:** a region between TADs is called **topological boundary** if its length is less than 400 kbp, otherwise it is called **unrecognized chromatin.**
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TAD borders: a region between TADs is called topological boundary if its length is less than 400 kbp, otherwise it is called unrecognized chromatin.

Topological boundaries in mouse ESC were found to be quite small, 76.33% of them being less than 50 kbp.
The main biological results in Dixon et al., 2012 are as follows:

- TADs were called in mouse and human ESC, as well as in some terminally differentiated cell types. E.g., about 91% of the mouse ESC is occupied by TADs with median size around 880 kbp.
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These results (and raw Hi-C data from the paper) are used in biological studies (see, e.g., Battulin et al., 2015, Rao et al., 2014, Van Bortle, 2014, Pope et al, 2014, Duggal et al., 2014, Kolovos et al., 2014, Zhao et al., 2013, Lu et al, 2013)
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- Pope et al, 2014 called TAD borders (without HMM) in human fibroblasts IMR90 in order to compare them to those previously called in Dixon et al., 2012 (higher resolution Hi-C data were used) and to use them in replication-timing studies.
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- **Dileep et al., 2015** calculated DI in six regions at several time points in the G1-phase of mouse mammary epithelial cell line (C127) watching a switch from a negligible to strong directionality bias that suggested formation of TADs.
Insulation score
Insulation score

\[ L \]

\[ m_1, m_2, \ldots, m_k, i, n_1, n_2, \ldots, n_k \]
Insulation score (IS) is defined for a bin as an average number of interactions that occur across this bin in some vicinity of the bin (Crane et al., 2015):

\[
\text{IS} = \frac{1}{k^2} \sum_{m \in M, n \in N} C(m, n),
\]

where \( N = \{n_1, n_2, \ldots, n_k\} \), \( M = \{m_1, m_2, \ldots, m_k\} \), \( C(m, n) \) is a number of interactions between bin \( m \) and bin \( n \).
We expect that IS has local minimums at TAD borders.
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IS plot is often called **insulation profile**.
Insulation score

IS can be calculated using a square window sliding along the diagonal of a contact matrix: average number of interactions in this window is the insulation score value (Crane et al., 2015, adapted):
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IS calculation scheme (Crane et al., 2015):
TAD calling with IS (Crane et al., 2015):

- Calculate IS along a chromosome.

\[
\text{IS}_{\text{avg}} = \frac{\sum \text{IS}}{\text{Number of bins}}
\]

\[
\Delta \text{IS}_{i} = \log_2 \left( \frac{\text{IS}_{i}}{\text{IS}_{\text{avg}}} \right)
\]
TAD calling with IS (Crane et al., 2015):

- Calculate IS along a chromosome.
- Normalize each IS value: $\text{IS} := \log_2 \frac{\text{IS}}{\text{IS}_{\text{avg}}}$, where $\text{IS}_{\text{avg}}$ is the mean of all IS values for the chromosome.
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- Calculate $\Delta$ values for each bin $i$ (Crane et al., 2015, Extended Data):
Insulation score

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- Normalize each IS value: $IS := \log_2 \frac{IS}{IS_{\text{avg}}}$, where $IS_{\text{avg}}$ is the mean of all IS values for the chromosome.
- Calculate $\Delta$ values for each bin $i$. $\Delta_i = 0$ at all IS peaks and valleys (minimums) (Crane et al., 2015, adapted):

![Local minima determination](image)
**Insulation score**

**TAD calling with IS (Crane et al., 2015):**

- Calculate IS along a chromosome.
- Normalize each IS value: $\text{IS} := \log_2 \frac{\text{IS}}{\text{IS}_{\text{avg}}}$, where $\text{IS}_{\text{avg}}$ is the mean of all IS values for the chromosome.
- Calculate $\Delta$ values for each bin $i$. $\Delta_i = 0$ at all IS peaks and valleys (minimums) (Crane et al., 2015, adapted):
  - TAD border is called at bin $i$ if $\Delta_i = 0$, the nearest $\Delta$ local max ($\Delta_{\text{max}}$) is to the left of bin $i$, the nearest $\Delta$ local min ($\Delta_{\text{min}}$) is to the right, and $S_i \equiv \Delta_{\text{max}} - \Delta_{\text{min}} > 0.1$. $S_i$ is called **border (boundary) strength**. TAD is called between two borders.
Crane et al., 2015 published their Perl script for TAD calling with IS.
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Barutcu et al., 2015 called TADs with IS to see differences in higher order chromatin structure between MCF-10A mammary epithelial and MCF-7 breast cancer cell lines.
Contrast index

\[ L \]

\[ i \]

\[ L \]
Contrast index
Contrast index is defined as follows (Van Bortle et al., 2014, Alekseyenko et al., 2015):

\[ CI = \frac{A + B}{C}, \]

where \( A \) is a total number of interactions to the left of bin \( i \) in \( L \)-vicinity, \( B \) is a total number of interactions to the right of bin \( i \) in \( L \)-vicinity, and \( C \) is a number of interactions that occur over bin \( i \) from the left \( L \)-vicinity to the right.
CI calculation using a contact matrix (fig. is based on Crane et al., 2015):
Contrast index

CI calculation using a contact matrix (fig. is based on Crane et al., 2015):

\[ CI = \frac{\sum_A + \sum_B}{\sum_C}, \]

where \( \sum_A, \sum_B, \sum_C \) are sums of elements in A, B, and C contact submatrices, respectively.

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Contrast index

- TAD is called between two bins with CI values higher than some threshold.
Contrast index

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- Van Bortle et al., 2014 studied a relationship between TAD border strength and architectural proteins binding site (APBS) abundance (fig. is adapted):
Contrast index

- CI was used for TAD calling and TAD border strength assessment in several papers. E.g.:
  - Li et al., 2015 studied TAD border strength decline in *Drosophila* cells after heat-shock:
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Conclusion

- **TADs** are stable and evolutionary conserved units of transcription regulation in mammals. Some similar self-interacting domains were found in other Eukaryotic species.
**Conclusion**

- **TADs** are stable and evolutionary conserved units of transcription regulation in mammals. Some similar self-interacting domains were found in other Eukaryotic species.
- **Pros** and **cons** of considered TAD calling methods:

  - **DI**, **IS**, and **CI** are intuitive and inferred directly from TAD definition. They can be used both for TAD calling and TAD border strength assessment.
  - **DI**, **IS**, and **CI** are easy to compute: each of them can be calculated in $O(NK)$ time for one chromosome, where $N$ is a number of bins in a chromosome, and $2^K$ is a number of bins in the $2^L$-vicinity of each bin. Typically, $K$ is much less than $N$.
  - We need an arbitrary threshold or percentile or a kind of HMM to call TADs with these metrics.
  - There are almost no published and well-tested tools for TAD calling using these metrics.
  - **DI**, **IS**, and **CI** can't enable us to call a TAD hierarchy (a TAD with its sub-TADs) as a whole.
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  - DI, IS, and CI are easy to compute: each of them can be calculated in $O(NK)$ time for one chromosome, where $N$ is a number of bins in a chromosome, and $2K$ is a number of bins in the $2L$-vicinity of each bin. Typically, $K$ is much less than $N$.
  - We need an arbitrary threshold / percentile or a kind of HMM to call TADs with these metrics.
  - There are almost no published and well-tested tools for TAD calling using these metrics.
  - DI, IS, and CI can’t enable us to call a TAD hierarchy (a TAD with its sub-TADs) as a whole.
Conclusion

**Pros** and **cons** of **considered methods**:

- DI, IS, and CI are intuitive and inferred directly from TAD definition.
- They can be used both for TAD calling and TAD border strength assessment.
- DI, IS, and CI are easy to compute: each of them can be calculated in $O(NK)$ time for one chromosome, where $N$ is a number of bins in a chromosome, and $2K$ is a number of bins in the $2L$-vicinity of each bin. Typically, $K$ is much less than $N$.
- We need an arbitrary threshold / percentile or a kind of HMM to call TADs with these metrics.
- There are almost no published and well-tested tools for TAD calling using these metrics.
- DI, IS, and CI can’t enable us to call a TAD hierarchy (a TAD with its sub-TADs) as a whole.

**In Part 2** I’ll consider *some* of the following much more complicated methods and tools for TAD calling: Sexton et al., 2012; Hou et al., 2012; Armatus, 2014; HiCseg, 2014; Arrowhead algorithm, 2014; TADtree, 2015; TADbit.
Chromatin conformation overviews

Self-interacting chromatin domains in various species


Chromatin conformation capture methods:


- **Some Hi-C derivatives:**
Hi-C data processing and analysis

Overviews:


Hi-C data correction:

TAD calling methods

Covered in this overview:


Thank you!

Sam Rose. Epigenetics and organisation