

# HiC Contact Map Comparison Using Graphlet Approach

Behnam Rasoolian<sup>1,\*</sup>, Debswapna Bhattacharya<sup>1,\*</sup>

<sup>1</sup> Auburn University

## ABSTRACT

**In this study, we investigated dissimilarities between normal cells and cancerous cells, through analyzing HiC contact maps. Our results show that certain orbit distributions have significantly higher correlation between Leukemic cells.**

## INTRODUCTION

Graphlet comparison is a novel method used to compare large networks in order to find local similarities in them. Authors of (1) provide a new measure of PPI network comparison based on 73 constraints. This is used in order to compare two large networks in order to detect similarities.

(2) provide heuristics to compare two nodes based on some feature (or signature) vectors, which is a 73-dimensional vector  $\mathbf{s}^T = [s_0, s_2, \dots, s_{72}]$  where  $s_i$  denotes the number of nodes in the network that are part of an orbit  $i$ .

**Important Result:** Proteins with similar surroundings perform similar functions.

In (3), the same author investigates cancer-causing genes to find similarities in their signatures. After clustering the genes based on *signature similarity* criteria, some clusters contain a lot of cancerous genes. They use 4 different clustering methods with varying parameters to cluster the proteins. They then predict the cancer-relatedness of a protein  $i$  using an enrichment criteria  $\frac{k}{|C_i|}$  where  $C_i$  is the cluster where protein  $i$  belongs and  $k$  is the number of cancer-causing proteins in  $C_i$  and  $|C_i|$  is the size of  $C_i$ .

The authors of (4) generalized the idea of graphlets to ordered graphs where the nodes are labeled in ascending order. As can be viewed, there are a total of 14 orbits for graphlets of size 2 and 3 since the label of graphlets is also included in topology. In the new definition,  $d_v^i$  denotes the number of orbit  $i$  touches node  $v$ . Each node, is then assigned a vector of length 14<sup>1</sup> ( $d_v^1, d_v^2, \dots, d_v^{14}$ ) and similarity of two nodes in two contact maps can be compared by how geometrically close their corresponding vectors are.

**Notations** In this paper, matrices and vectors are represented with bold capital and bold small letters respectively. matrix rows and columns are represented by a *dot* notation. For

example, the  $i$ th row of matrix  $M$  is denoted by  $M_{i\cdot}$  and its  $j$ th column is represented by  $M_{\cdot j}$ .

We denote the set of all contact maps in cell line  $T$  with  $\mathbb{C}^T$ . If no particular cell line is addressed, the subscripts are dropped. Any arbitrary member of  $\mathbb{C}$  is denoted by  $C_{ij}$ , where  $i$  and  $j$  ( $j \geq i$ ) represent the two chromosomes involved. In human cells this set contains a total of 276 contact maps, 23 of which are intra-chromosomal and the rest are inter-chromosomal. For ease of representations, intra-chromosomal contact maps are distinguished by a single superscript, so we have  $C_{i,i} = C_i$ .

We denote the number of loci in a chromosome  $i$  by  $N_i$ . The set of all loci involved in contact map  $C_{ij}$  is denoted by  $\mathbb{V}_{ij}$ . In intra-chromosomal contact maps,  $\mathbb{V}_{i,i}$  contains only the loci of that particular chromosome ( $|\mathbb{V}_i| = N_i$ ), while in inter-chromosomal contact maps  $\mathbb{V}_{ij}$  contains the loci in the both of chromosomes involved ( $|\mathbb{V}_{ij}| = N_i + N_j$ ).

## MATERIALS AND METHODS

We re-used Leukemic Hi-C libraries created in (5) These libraries we sequenced for cases of primary human B-acute lymphoblastic leukemia (B-ALL or ALL), the MHH-CALL-4 B-ALL cell line (CALL4), and the follicular lymphoma cell-line (RL). Just as (5), we used normal B-cell line (GM068990) from (6) for our comparisons. We created contact maps of resolution 500kb and normalized it using the *iced* package in python developed by (7).

### Thresholding contact maps

In order to be able to extract graphlets, HiC contact maps should be modeled as unweighted graphs where the nodes represent the loci and an edge between two nodes represent a *significant* interaction between the loci. This can be achieved by thresholding the contact maps. The result of the thresholding procedure is a binary matrix which also can serve as an adjacency matrix for an unweighted, undirected graph. The graph can then be used for orbit extraction.

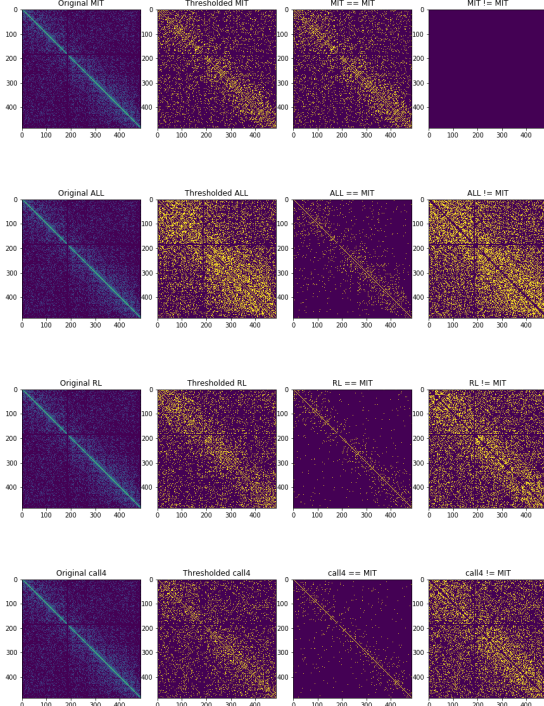
When thresholding contact maps, it is necessary to make sure that both global and local features are maintained. We could consider thresholding the contact maps by simply setting values above a fixed value to one and the rest to zero; However, in practice, this method resulted in graphs that capture the local structure of the contact maps poorly.

\*Tel: +1 334 5212814; Email: bzt0014@auburn.edu

<sup>1</sup>number of orbits in graphlets of size 2 and 3

© 2017 The Author(s)

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/2.0/uk/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.



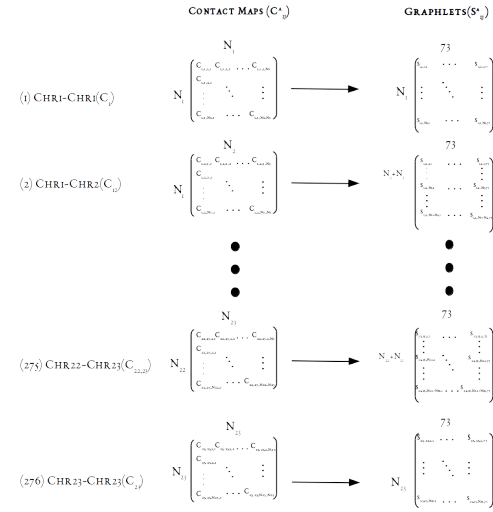
**Figure 1.** Result of thresholding interchromosomal contact map of chromosome 1 using a kernels of size  $5 \times 5$  for all cell lines. The first row shows the thresholded maps. Second and third rows demonstrate pair-wise similarities and differences between contact maps respectively.

This is because intensities follow an exponential distribution with a mean close to zero with a few very large values that correspond to interactions along or close to the main diagonal of the contact maps. Thus, picking relatively large numbers would result in ignoring interactions that are far from the main diagonal while picking small values will lead to capturing too many (insignificant) interactions.

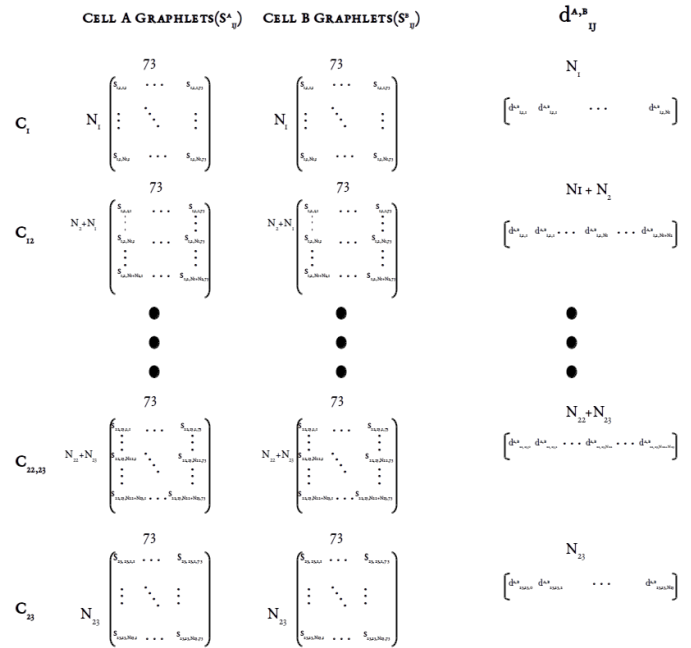
In order to threshold the matrix so that both global and local patterns are captured, we borrowed the concept of *adaptive thresholding* from image processing context. In this method, in order to be set, a pixel should have an intensity larger than the average of non-zero intensities in its *neighborhood*. The neighborhood is defined by a sliding kernel that passes through the contact map with the pixel at its middle at each step. Figure 1 demonstrates result of this thresholding approach for intra-chromosomal contact maps of chromosome 1. Refer to supplementary material for all 23 interchromosomal thresholding results.

### Orbit Extraction

Once the thresholded contact maps are obtained, graphlets and orbits can be extracted. We used the `orca` package in R programming language to extract the graphlets. As a result



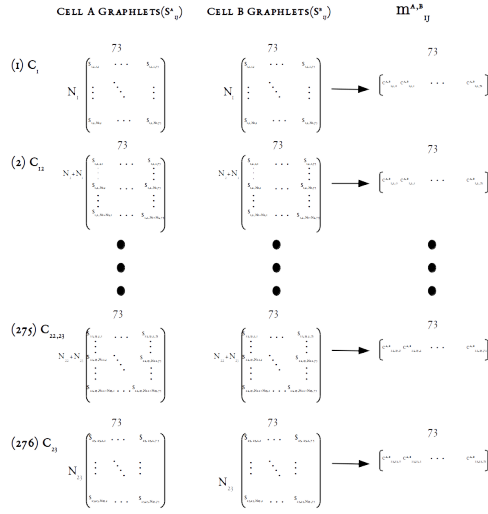
**Figure 2.** Graphlet extraction for the four cell lines. For each loci in each contact map between chromosomes  $i$  and  $j$ , the signature vectors of length 73 are extracted, resulting in a *signature matrix* of size  $|V^{ij}| \times 73$ , where  $V^{ij}$  is the number of loci involved.



**Figure 3.** Calculating pair-wise loci distances. For each loci (row) in each contact map in MIT cell line, its distance is calculated based on equation 1 with the corresponding loci in leukemic cells. The result of this process is a *signature distance vector* of size  $|V^{ij}| = N_i + N_j$  for each contact map.

of graphlet extraction, For each loci in each contact map, a *signature vector* of size 73 is created. Thus for each cell line, we would have 276 *signature matrices* of size  $|V^{ij}| \times 73$ , where  $V^{ij}$  is the number of loci involved in contact map between chromosomes  $i$  and  $j$ . Figure 2 illustrates the process and results of signature matrix extraction schematically.

For a particular  $C_{ij}$ , we denote  $S_{ij}$  as its *signature matrix*. Each cell  $S_{ijlo}$  in  $S_{ij}$  captures how many times loci  $l$  in  $C_{ij}$  occurred as part of orbit  $o$ .



**Figure 4.** Calculating pair-wise orbit correlations. For each orbit (column) in each contact map in MIT cell line, its correlation with the same orbit in the same contact map in leukemic cells is calculated. The result of this process is a *signature correlation* vector of size 73 which captures how similar frequencies of two orbits are. In order to test our second hypothesis, we calculated averages across contact maps (along the vertical red arrow) to test hypothesis ?? and across orbits (along the horizontal red arrows) to test hypothesis ??.

We consider two measures of *difference* when comparing contact map graphlets across cell lines. The first measure is *signature distance vectors* between each contact map of two cell lines. For a pair of cells A and B, let  $S_{ij}^A$  and  $S_{ij}^B$  be their signature matrices. The *signature distance* of contact map  $C_{i,j}$  between A and B is denoted by  $d_{ij}^{A,B}$ .  $d_{ij}^{A,B}$  is a vector of size  $|V_{i,j}|$  and its elements  $d_{i,j,l}^{A,B}$  are calculated using the following formula from (1):

$$d_{i,j,l}^{A,B} = \frac{1}{73} \sqrt{\sum_{o=0}^{72} t_{lo}^2} \quad (1)$$

where elements of  $t_{i,j,l,o}$  is the distance between each loci (row)  $l$  in  $S^A$  and the the same loci in  $S^B$  for orbit  $o$  as is calculated as below:

$$t_{lo} = w_o \times \frac{\log(S_{ijlo}^A + 1) - \log(S_{ijlo}^B + 1)}{\log(\max(S_{ijlo}^A, S_{ijlo}^B) + 2)} \quad (2)$$

This process is illustrated in Figure 3. Using this distance measure, we can quantify how two loci are close to each other in terms of local neighborhood between the two contact maps.

The second measure of comparison that we use captures how similar two orbits are in terms of their count frequencies across loci between two contact maps. Each column in  $S_{ij}$  can provide information regarding the *frequency distribution* of orbits throughout the contact map  $C_{ij}$ . We can find how similar these distributions are to each other using correlation

	One	MIT-RL	ALL-RL	ALL-MIT	ALL-CALL4	CALL4-MIT	CALL4-RL
One	-	>	>	>	>	>	>
MIT-RL	<	-	<	<	<	<	<
ALL-RL	<	>	-	<	<	<	<
ALL-MIT	<	>	>	-	=	=	=
ALL-CALL4	<	>	>	=	-	=	=
CALL4-MIT	<	>	>	=	=	-	-
CALL4-RL	<	>	=	=	=	=	-

**Table 1.** Result of one-sided t-test for intrachromosomal contact map of chromosome 1.  $d_{1,1}^{CALL4,MIT}$  is statistically larger than 0, but less than  $d_{1,1}^{ALL,RL}$  and so forth. We can also conclude that  $d_{1,1}^{ALL,CALL4}$  is not statistically different from  $d_{1,1}^{ALL,MIT}$  between graphlets extracted from all contact maps. Refer to supplementary material for results of all hypothesis tests.

measures. These correlations are denoted by  $m_{i,j}^{A,B}$  and can be calculate using any plausible correlation measure. In this study, for each contact map, we calculated similarity between orbit distributions using Pearson’s  $r$  correlation, which is computationally efficient. However, pearson’s  $r$  might not be able to capture non-functional relationships between distributions. As a result, we also used Maximal Information Coefficient (MIC) (8) in order to compare correlations. MIC calculates mutual information (MI) between two distributions, but utilizes dynamic programming in order adjust bin sizes and numbers in order to achieve highest MI. MIC values between two variables fall between 0 and 1, with 0 meaning the two variables are completely independent and 1 meaning one is dependant on the other. We used both Pearson’s  $r$  and MIC in order to compare orbit frequencies. Although results from both approaches were more or less consistent, MIC showed higher robustness than Pearson’s  $r$  method.

If MIC is used as correlation measure, each element of  $c$  is calculated as below:

$$m_{ijo}^{A,B} = MIC(S_{ij,o}^A, S_{ij,o}^B) \quad (3)$$

Alternatively, if we use Pearson criterion we would have:

$$m_{ijo}^{A,B} = Pearson(S_{ij,o}^A, S_{ij,o}^B) \quad (4)$$

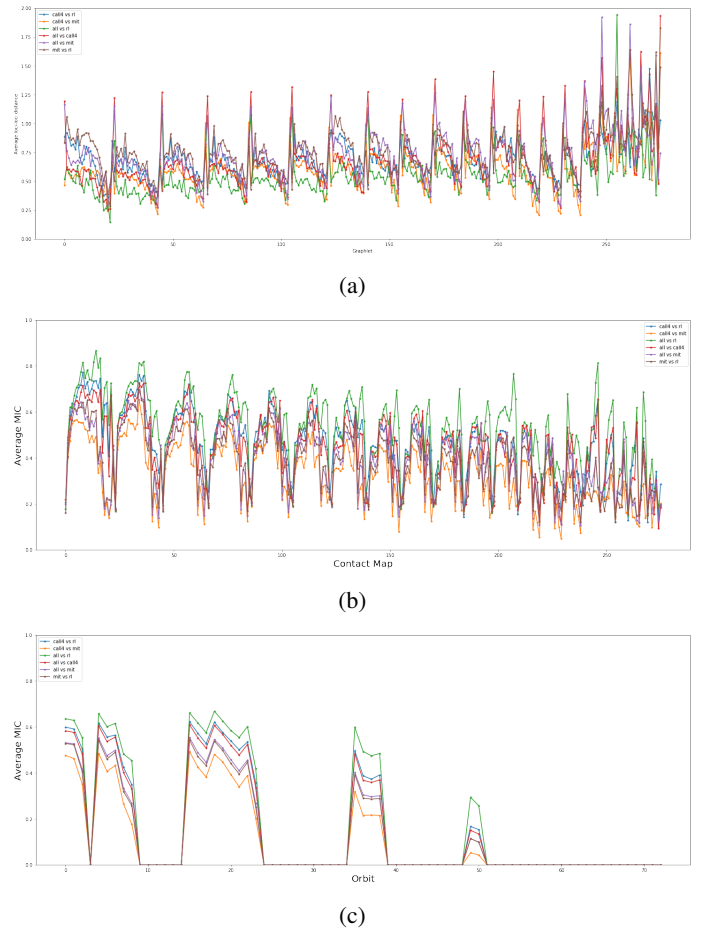
## RESULTS AND DISCUSSIONS

Result of pair-wise contact map graphlet distances is illustrated in Figure 5a. Each point on the graph is the average of the graphlet distance vector of the two cell lines specified in the legend ( $d_{i,j}^{A,B}$ ). A one sided paired t-test was conducted in order to compare distances to zero and also to each other. The resulting p-values showed highly significant differeces from zero for all pairs of cells. The results of tests for  $C_{1,1}$  is shown in table 1 Results of all t-tests for each contact map can be found in supplementary materials. For each contact map, each pair of cells are ordered based on whether they are statistically larger that the other or not with a signifiigance of 0.01.

We calculated pair-wise MIC for each orbit in each of the 276 contact maps from MIT data and ALL, RL and CALL4 data separately. Figure 5b shows average orbit correlations across all contact maps, while figure 5c demonstrates average correlations across all 72 orbits within each contact map. It is worth mentioning that interchromosomal thresholded contact maps represent a bipartite graph with the loci from each chromosome on one side. Due to this bipartite nature of the graphs in inter-chromosomal maps, count of certain orbits is always 0, resulting in a correlation values of 0 for them as well. We ignored these values when we calculated averages across orbits in figure 5b since they would result in a bias towards zero in averages. You can see the bias in figure 5c where average correlations of orbits  $\mathbb{Q} = \{3, 9, 10-14, 20-34, 39-48, 51-72\}$  are close to zero. In fact all correlations corresponding to these orbits are 0 except for the ones between the same chromosomes.

Figure 5c clearly illustrates certain orbits of Leukemic have higher correlation to each other than to the normal MIT cell. In fact our statistical analysis shows that *for orbits in  $\mathbb{Q}$ , intra-leukemic orbit correlations are significantly higher than leukemic-normal orbit correlations*. This implies there are significant differences between normal and leukemic cells in terms of their local structure.

We have conducted one-sided t-test in order to test whether the average correlations across contact maps is equal to 1 and whether the average correlations across orbits is equal to 1. The results for both test showed that all values are significantly less than 1. Please refer to supplementary material for result of the full list of t-test results.



**Figure 5. (a) Pair-wise graphlet signature difference for all 276 contact maps:** Each point on a graph is the result of averaging all the distances across all loci of that contact map. ( $\bar{d}_{i,j}^{A,B} \forall i,j \in \{1...23\} \ \& \ j \geq i$ ).

**(b) pair-wise average contact map orbit correlations for all contact maps:** ( $\bar{m}_{i,j}^{A,B} \forall i,j \in \{1...23\} \ \& \ j \geq i$ : average along the red vertical arrow in figure 4). These values are calculated by averaging over pairwise correlations of orbits of  $\mathbb{Q}$  in a contact map.

**(c) pair-wise average orbit correlations:** In figure 5c, each point in the graph is the result of averaging pair-wise orbit correlations over all contact maps ( $\frac{1}{276} \sum_{i=0}^{23} \sum_{j=i}^{23} m_{i,j,o}^{A,B} \ \forall o \in \{0,1,...,72\}$ : average along the red horizontal arrows in figure 4). Counts for certain orbits are always zero in inter-chromosomal maps, leading to average value close to zero in Figure 5c.

## RESOURCES

### Hi-C Datasets:

1. Code base for this article
2. Datasets including cancerous cells
3. Original Datasets

## REFERENCES

1. Nataša Pržulj. Biological network comparison using graphlet degree distribution. *Bioinformatics*, 23(2):e177–e183, 2007.
2. Tijana Milenković and Nataša Pržulj. Uncovering biological network function via graphlet degree signatures. *Cancer informatics*, 6:257, 2008.
3. Tijana Milenković, Vesna Memišević, Anand K Ganesan, and Nataša Pržulj. Systems-level cancer gene identification from protein interaction

- network topology applied to melanogenesis-related functional genomics data. *Journal of the Royal Society Interface*, 7(44):423–437, 2010.
4. Pietro Di Lena, Piero Fariselli, Luciano Margara, Marco Vassura, and Rita Casadio. Fast overlapping of protein contact maps by alignment of eigenvectors. *Bioinformatics*, 26(18):2250–2258, 2010.
  5. Zheng Wang, Renzhi Cao, Kristen Taylor, Aaron Briley, Charles Caldwell, and Jianlin Cheng. The properties of genome conformation and spatial gene interaction and regulation networks of normal and malignant human cell types. *PloS one*, 8(3):e58793, 2013.
  6. Erez Lieberman-Aiden, Nynke L Van Berkum, Louise Williams, Maxim Imakaev, Tobias Ragoczy, Agnes Telling, Ido Amit, Bryan R Lajoie, Peter J Sabo, Michael O Dorschner, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *science*, 326(5950):289–293, 2009.
  7. Nicolas Servant, Nelle Varoquaux, Bryan R Lajoie, Eric Viara, Chong-Jian Chen, Jean-Philippe Vert, Edith Heard, Job Dekker, and Emmanuel Barillot. Hic-pro: an optimized and flexible pipeline for hi-c data processing. *Genome biology*, 16(1):259, 2015.
  8. David N Reshef, Yakir A Reshef, Hilary K Finucane, Sharon R Grossman, Gilean McVean, Peter J Turnbaugh, Eric S Lander, Michael Mitzenmacher, and Pardis C Sabeti. Detecting novel associations in large data sets. *science*, 334(6062):1518–1524, 2011.