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Title: Single-cell RNA-seq does not yield a relationship in gene pair distance versus correlation

Single-cell RNA-seq does not yield a relationship in gene pair distance versus correlation

Jason Yang, Martin Falk, Sameer Abraham

Abstract:

In some organisms such as E. coli and S. cerevisiae yeast, it is known that there is a relationship between the distance among genes and their coexpression (Pannier et. al., Kruglyak and Tang). It is also known that in general there is a relationship between gene function and genome structure (Szabo et. al). One might also expect to find a relationship between gene expression and TADs, which are domains within the genome where loci inside contact each other more frequently than loci outside. However, by analyzing data from murine brain cells, we do not find a relationship between gene pair correlation of single-cell RNA-seq gene expression and gene pair distance. Furthermore, despite the body of work linking gene expression and TAD structure, we also find no difference between gene pairs within a single TAD and between two TADs in terms of the relationship between gene pair distance and correlation.

Keywords:

TAD, Hi-C, RNA-seq, correlation

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Introduction:

In some organisms such as E. coli and S. cerevisiae yeast, it is known that there is a relationship between the distance among genes and their coexpression (Pannier et. al., Kruglyak and Tang). It is also known that in general there is a relationship between gene function and genome structure (Szabo et. al). One might also expect to find a relationship between gene expression and TADs, which are domains within the genome where loci inside contact each other more frequently than loci outside. We investigate if these relationships hold in mus musculus neurons.

Single-cell RNA sequencing measures the activity of all genes in a variety of cell types. The data is collected by isolating the desired cells, converting RNA strands into cDNA strands, and counting the frequency of genes in those strands. To make this counting practical, the cDNA is amplified, or duplicated many times, using techniques such as PCR (Luecken et. al.)

TADs, or topologically associating domains, are regions of DNA where genes interact with each other more often inside than outside of (Szabo et. al.) They are shown in Hi-C as small, bright squares along the diagonal within a chromosome. For background, Hi-C is a form of

chromosome conformation capture that measures how often pairs of DNA loci contact. First, physically close pairs of DNA loci are bonded together, or crosslinked, by chemicals such as formaldehyde. The non-bonded areas are then cut out with enzymes, leaving only the crosslinked areas. The two strands of each piece of crosslinked DNA are joined, or ligated, into a single strand, and the crosslinkers are removed. This leaves small strands of DNA each coming from two separate locations in the original DNA. (Fig. 1) These strands are sequenced to count how often pairs of loci have been crosslinked. The sequencing results estimate how likely any two loci on DNA are to contact each other within the nucleus. (van Berkum et. al.)

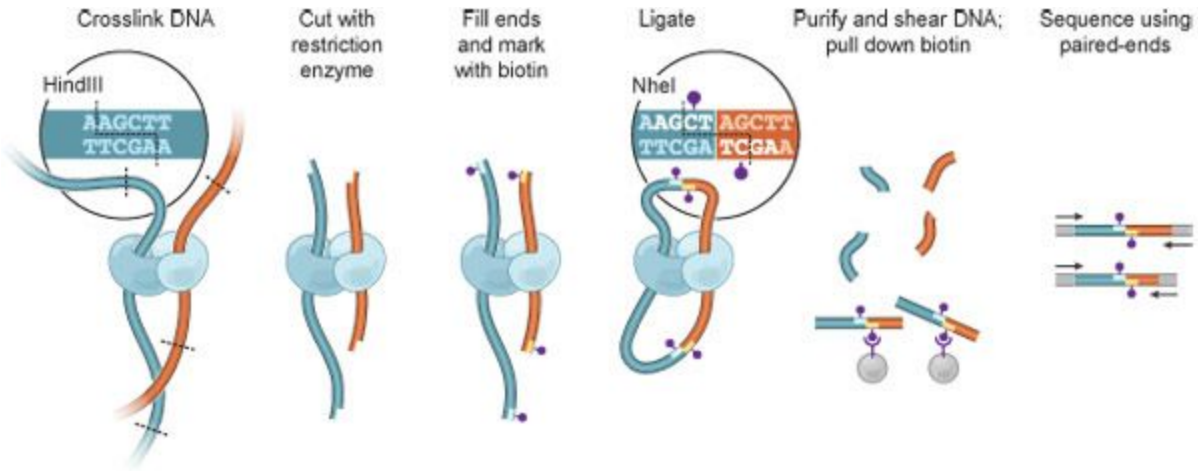

Figure 1: Description of Hi-C as a chromosome conformation capture method, a way of measuring how often any two DNA loci contact each other in the nucleus (van Berkum et. al.). Places on DNA that are close together are first bound, or crosslinked, with chemicals such as formaldehyde. The pieces of DNA not crosslinked are then cut away. The loose ends of each pair of crosslinked DNA loci are ligated together before the crosslinking chemical is removed, and the resulting DNA pieces are sequenced.

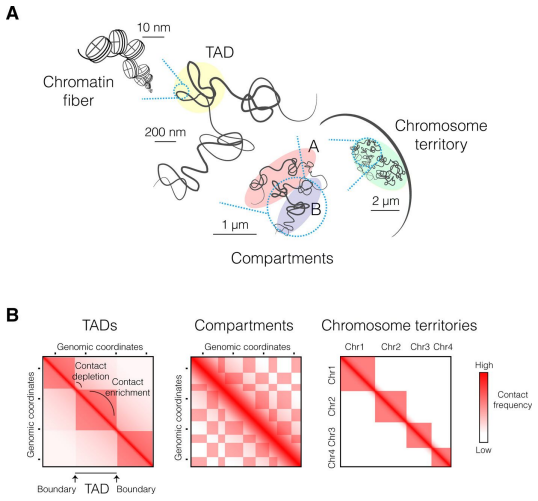

Figure 2: TADs are small areas of DNA where loci within a single TAD contact each other more frequently than loci between two TADs. Compartments are larger groups of loci, spread across the genome, where loci in the same compartment contact each other more frequently than loci from two different compartments (it is theorized that there are mainly two kinds of compartments). Chromosome territories are chromosomes themselves, since loci within a single chromosome contact each other much more frequently than loci across two chromosomes. TADsare generally smaller than compartments, which are smaller than chromosome territories (Szabo et. al.).

Distance and Correlation:

We acquired the normalized counts of single-cell RNA-seq data from He et. al. and did not remove any outliers. We first investigated the relationship between the distance and correlation of pairs of genes within a single chromosome. Here we define the distance between two genes to be the distance between their centers and define the center of a gene to be the average of the positions of its endpoints. In other words, if gene A has endpoints at a_0 and a_1, and gene B has endpoints at b_0 and b_1, the distance between the two genes if $|(a_0+a_1)/2 - (b_0+b_1)/2|$. We define the correlation between two genes to be the Pearson correlation of the number of times both genes are expressed across all cells. Figure 3 shows that the average correlation stays relatively constant at around 0 as distance increases.

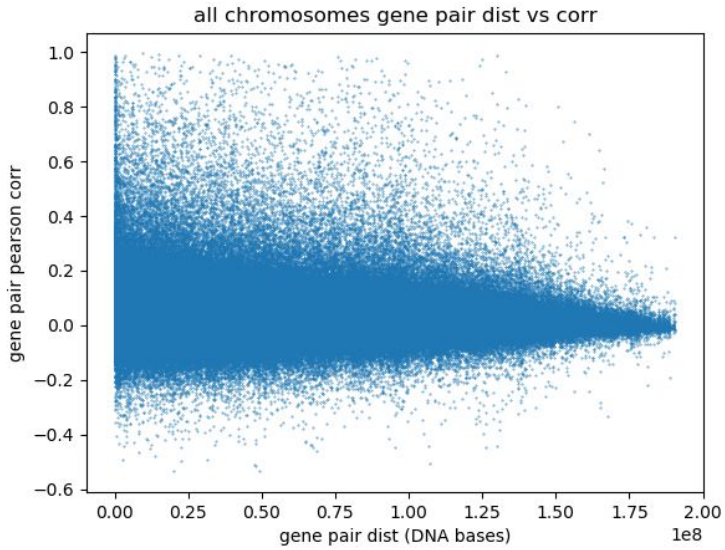

Figure 3: Distance vs. correlation between all pairs of genes within a single chromosome, for all chromosomes. Here the distance between two genes is defined as the distance between their centers, and define the center of a gene to be the average of the positions of its endpoints; the correlation between two genes is defined as the Pearson correlation of the number of times both genes are expressed across all cells.

Since there is a hypothesis that there is a relationship between gene activity and genome folding, specifically that of TADs, we looked to see if accounting for TADs would reveal any interesting results regarding the distance-to-correlation relationships. We got a list of TADs of retinal neurons from Falk et. al. and compared gene pairs that were within a single TAD (intraTAD) to pairs across two different TADs (interTAD). In the violin plot below, which only shows gene pairs with distance less than 500,000 DNA bases, the interTAD distributions of gene pair correlation are in light blue and the intraTAD distributions of gene pair correlation are in blue. There is little difference between the distance-to-correlation relationship when computed over gene pairs within a single TAD versus gene pairs across two TADs (Fig. 4).

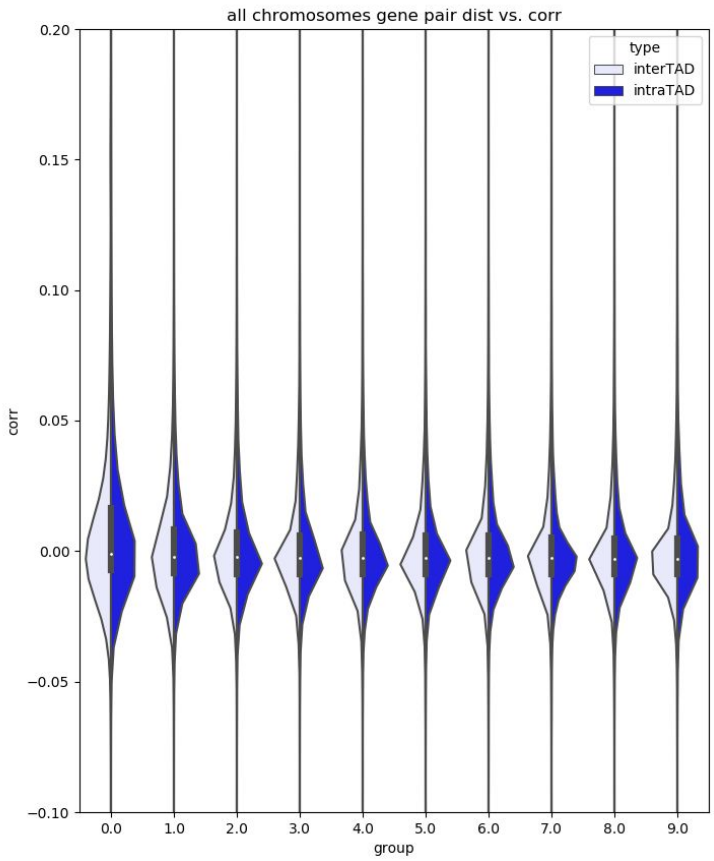

Figure 4: Violin plot of gene pair distance vs. RNA-seq correlation along all cells, with distance binned by 50,000s; only gene pairs with distance <500,000 are shown. Each violin shows the distribution of gene pairs across two TADs on the left (interTAD), and gene pairs within a single TAD on the right (intraTAD).

Finally, we accounted for different cell types among the cells in the RNA-seq data. These cell types were determined by He et. al. using BackSPIN clustering on the RNA-seq data, choosing the 6th split level.

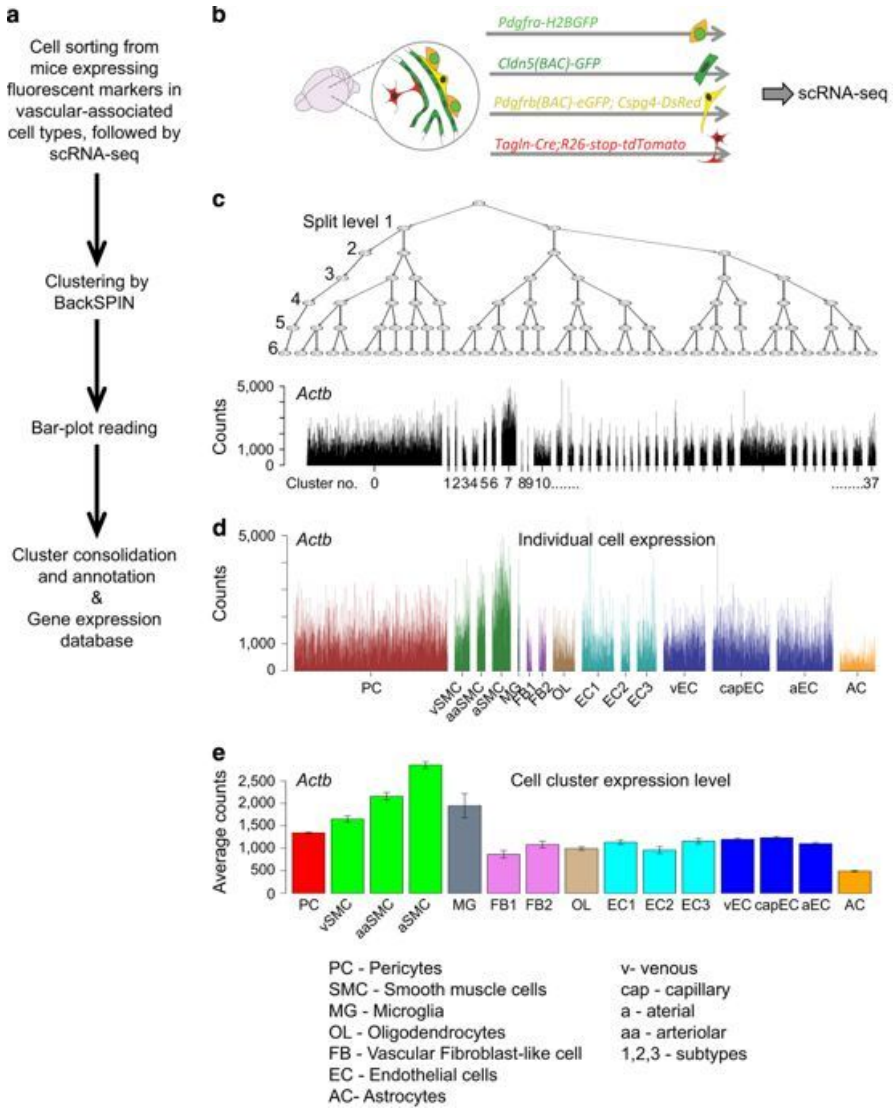

Figure 5: Derivation of cell types from He et. al. using the RNA-seq data. The authors used BackSPIN to cluster the cells based on the gene expression data, decided on choosing the 6th split level, and applied manual inspection to determine that there were 15 cell clusters.

We repeated Figure 4 but instead used correlation values between genes on RNA-seq data on only the cells from a single cluster. We show two such plots based on the two largest cell clusters. Again, there is little difference between gene pairs within a single TAD and across two TADs in terms of the distance-to-correlation relationship (Fig.s 6, 7).

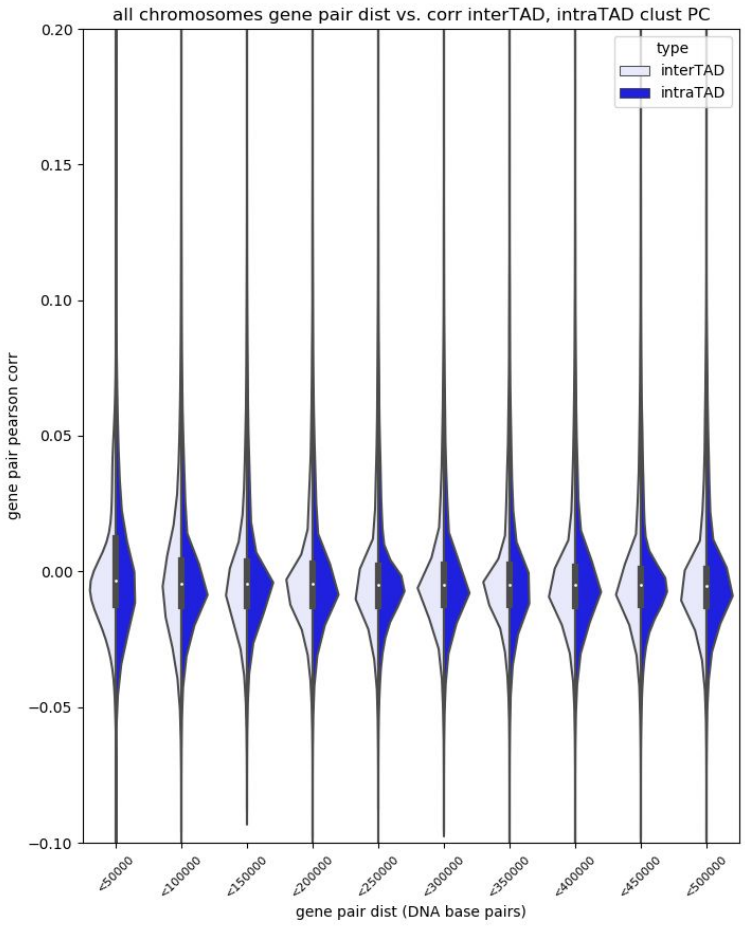

Figure 6: Violin plot of gene pair distance vs. RNA-seq correlation among cells of type PC (pericytes), determined by He et. al., the cell type containing the largest number of cells, with distance binned by 50,000s; only gene pairs with distance <500,000 are shown. Each violin shows the distribution of gene pairs across two TADs on the left (interTAD), and gene pairs within a single TAD on the right (intraTAD).

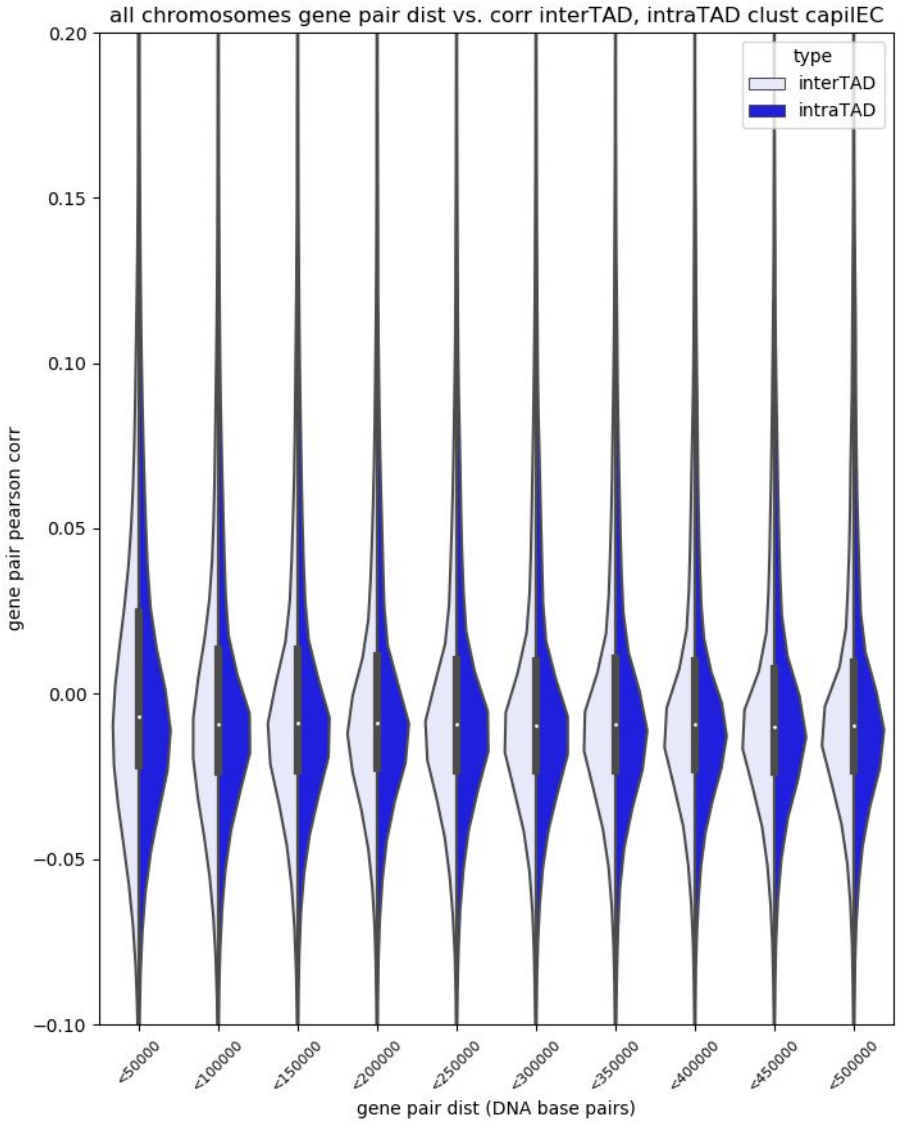

Figure 7: Violin plot of gene pair distance vs. RNA-seq correlation among cells of type capilEC (capillary endothelial cells), determined by He et. al., containing the second largest number of cells, with distance binned by 50,000s; only gene pairs with distance <500,000 are shown. Each violin shows the distribution of gene pairs across two TADs on the left (interTAD), and gene pairs within a single TAD on the right (intraTAD).

Conclusion:

Because there is a relationship between gene function, structure, and coexpression, in organisms such as E. coli and S. cerevisiae, we looked to see if the same relationship is present in murine cells. However, we did not find a relationship between gene pair correlation of single-cell RNA-seq gene expression and gene pair distance. Additionally, there was no change in this result when considering gene pairs within a single TAD versus those across two TADs. In the future,

we will redo this experiment with other gene sequencing data, such as single-cell ATAC-seq.

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