

1 **TReNCo: Topologically associating domain (TAD) aware regulatory network construction**

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30 **Abstract:**

31 There has long been a desire to understand, describe, and model gene regulatory networks
 32 controlling numerous biologically meaningful processes like differentiation. Despite many
 33 notable improvements to models over the years, many models do not accurately capture subtle
 34 biological and chemical characteristics of the cell such as high-order chromatin domains of the
 35 chromosomes. Topologically Associated Domains (TAD) are one of these genomic regions that
 36 are enriched for contacts within themselves. Here we present TAD-aware Regulatory Network
 37 Construction or TRenCo, a memory-lean method utilizing epigenetic marks of enhancer and
 38 promoter activity, and gene expression to create context-specific transcription factor-gene
 39 regulatory networks. TRenCo utilizes common assay's, ChIP-seq, RNA-seq, and TAD boundaries
 40 as a hard cutoff, instead of distance based, to efficiently create context-specific TF-gene
 41 regulatory networks. We used TRenCo to define the enhancer landscape and identify
 42 transcription factors (TFs) that drive the cardiac development of the mouse. Our results show
 43 that we are able to build specialized adjacency regulatory network graphs containing
 44 biologically relevant connections and time dependent dynamics.

Introduction:

It is of critical importance to understand, model, and describe gene regulatory networks (GRN) that control diverse cellular functions of interest like those that drive differentiation or transitions from one development stage to another (Lee et al. 2002; DeRisi et al. 1997; Goode et al. 2016). With the advent of next generation sequencing technologies, it is now commonplace to reconstruct these networks to connect transcription factors (TFs) to the genes they regulate (Karlebach and Shamir 2008). One classic method is integration of cis-regulatory elements, like enhancers, and gene expression via matrix factorization to form network graphs between genes and TFs (Marbach et al. 2016). Generally, this is done using Chromatin Immunoprecipitation (ChIP) for H3K27ac to identify enhancers and RNA-seq to identify controlled genes. In many cases connections are determined through perturbations in upstream components like TFs and observing resultant changes in downstream expression levels (Gasperini et al. 2019). This method works exceptionally well for certain classes of TF and for closely linked enhancer-gene interactions. However, it commonly uses arbitrary length cut offs to prevent enhancers from erroneously influencing genes in distant parts of the genome. This can lead to enhancers having shorter or broader ranges of influence than what occurs biologically. As many recent chromosome-conformation-capture (e.g. 5C, Hi-C and ChIA-PET) experiments have shown, there can be very broad and dynamic interactions made between different parts of a chromosome (Branco and Pombo 2007; McCord et al. 2020). Thus, it is more relevant to dynamically limit enhancers range of influence to only the topologically linked portions of the genome an enhancer is confined to, also known as Topologically Associated Domains (TADs). These regions are highly conserved across cell types and are known to limit

the influence of cis-regulatory elements by physically separating them (Beagan and Phillips-Cremens 2020). Thus, it is critical that these cutoffs are included in the model to fully represent and capture the true biological processes occurring.

Here we present TAD-aware Regulatory Network Construction or TReNCo, a powerful, memory efficient tool for constructing regulatory networks from enhancer, promoter, and gene expression data without the need for perturbations. We designed TReNCo to construct a graph of interaction weights between TFs and the genes that they control using TAD boundaries to dynamically limit the range of enhancer influence. We utilize dynamic programming to factor matrices within TADs and combine network into a full adjacency matrix for a regulatory graph. With this method, we are able to capture biologically relevant interactions between known TFs and their gene targets. We show that this network contains many subtle interactions that could be a treasure trove of novel or uncharacterized interactions. We believe this method opens the possibility for understanding deeper mechanistic connections and new possibilities for identifying biological targets for drug discovery.

Results and Discussion:

Model Design and Function

We designed TRenCo utilizing a previously reported core matrix factorization method with a distance-based scoring system broken down into subunits based on TAD boundaries (Marbach et al. 2016; Cuellar-Partida et al. 2012). In brief, our algorithm uses normalized gene expression count tables from RNA-seq (tsv files) and H3K27ac ChIP-seq read alignments (bam files), peaks (bed files), and TAD boundaries (a bed file) (Figure 1). Though the source of these data can vary, we designed TRenCo with ENCODE uniform processing pipelines in mind. We first generated initial expression matrices for gene expression (G) and enhancer expression (E) by sample. This was accomplished using the count tables from RNA-seq and building count tables for all enhancers merged into non-overlapping segments from the ChIP-seq data. These counts were used to calculate the Transcripts/Fragments per Kilobase Million (TPM) which are then log-scaled. A key file, provided by the user, is used to link related files to build a full expression matrix and, secondarily, serves to reduce memory usage by allowing batch processing of data.

We next worked to establish TF-gene linkages by identifying TF binding sites in promoters and enhancers using a well-known program, FIMO (a part of the MEME software suite and report the log-odds score of TF binding) a major weight needed for establishing interaction (Grant et al. 2011). We designed a simple pipeline to generate promoter and enhancer master bed files and remove any potential overlaps between promoters and enhancers to ensure that TFs are not double counted to a gene. Furthermore, these files contained the union of all promoters and enhancers between the samples in order to

streamline the identification of TFs. This was a critical time saving step as FIMO cannot be multithreaded and can take upward of 24 hours to run. By using master files, we could run FIMO only once per process leading to a huge performance boost.

With these core datasets, for each sample we were able to select sample-specific genes, enhancers, and TF interactions. To ensure proper TAD boundaries were followed and to improve speeds through multithreading, we designed a dynamic programming algorithm to process these datasets by TADs and generate TAD-specific distance weight matrices (D^t) for each set. These matrix subsets were factored with the square-root of a TAD-specific interaction matrix produced via vector multiplication between the gene ($g^{k,t}$) and enhancer ($e^{k,t}$) expression profiles resulting in a TAD-weight matrix ($W^{k,t}$). To generate an enhancer-specific graph edges, the weight matrix was factored with the TAD-specific enhancer-TF by gene matrix (M^t) normalized to the maximum value of the matrix. This was done to set a standard scale of log-odds that was comparable between enhancers and promoters. We designed this component with the assumption that enhancer-TF binding should be similar in promoters and should be weighted the as a log-odds scale in the network. A promoter-TF by gene specific subnetwork (P_t) was produced in a similar manner as the enhancer-specific network with weighing done using a TAD-specific gene expression vector since all distances between promoters and genes are 1. Arctangents were applied to both matrices due to the properties of the transformation where larger values approach an asymptote of $\pi/2$ while smaller values are approximately scaled linearly. This scaling draws larger value outliers into a tighter range without heavily influencing lower values and assumes a maximum impact a TF can have on a gene. The resulting matrices were added together and further weighted by normalized TF gene expression to lower

the influence of lowly expressed TFs while minimally changing the effects from highly expressed TFs. The resulting TAD subgraphs were concatenated together into a full network adjacency graph matrix. Since this was a sparse matrix, TReNCo represents it as an adjacency array allowing us to store the information in much less space than is needed for a matrix.

Model Validation

To validate the model, we used the extensive cohort of matched gene expression and H3K27ac ChIP-seq analyses in ENCODE (Davis et al. 2018) (Table S1). We decided to use mouse heart data due to the abundance of well correlated time point data spanning embryonic day 10.5 to 8 weeks after birth, highly characterized heart developmental processes, and the availability of previously documented TF-gene networks (Akerberg et al. 2019; Schlesinger et al. 2011) (Figure 2). While the ChIP-seq data is not highly correlated across all the sample types, the gene expression data has an R-squared of at least 0.7 between different biological samples. One e14.5 experiment set had an average R-squared of approximately 0.6 with all other biological samples. To remove this potentially problematic dataset in this analysis before the larger more computationally expensive processes occur, we added an optional soft filter in TReNCo to automatically remove any samples with an average R-squared less than 0.7 across all samples, for gene expression data. We were left with a set of highly correlated data that led us to conclude that this dataset was sufficient to use to TReNCo.

Previous studies of the mouse heart have identified Gata4, Mef2a, Nkx2-5, Tbx5, and Srf as important embryonic lethal TFs critical for development (Gittenberger-De Groot et al. 2005). When looking at the distribution of these TFs over time, we observed that there are many subtle dynamics in how the TFs' weights shift. Gata4, Mef2a, Tbx5, and Nkx2-5 show a

multimodal distribution with three major peaks and varying differences between time points though mostly the distributions overlapped (Figure 3A, Figures S2, S3, S4). We found that the weight distribution followed a similar trend; the dominant population of edge weights appears less than 0.1, a second mid population is between 0.1 and 0.3, and a final population above 0.3 that stretches up to 1. Another TF, Foxs1, demonstrated a more pronounced time point dependent change in addition to a tri-modal edge score (Figure 3B). Interestingly, Srf did not show this trend and tended to have lower weight edges throughout the distributions. To visualize the timepoint dynamics more clearly, we generated a heatmap of the distributions with inflection points added to determine changes in gene weights that may occur (Figure 3C and D). Inflection points, in this case, are simple differences in weights between each time point and the previous time point. These data are ideal for highlighting changes between each time point. An additional differential heatmap of all weight differences with respect to the embryonic day 10.5 point was generated to visualize change from a central time (Figure S1). It was clear that these TFs have time dependent dynamics in our model. Gata4, Nkx2-5, and Tbx5 appear to interact with most of their targets constantly throughout early development as indicated by a mostly yellow (no change) inflection point heat map until adult heart. These TF's have been shown to be important in normal cardiac development (Misra et al. 2014) and act as potential as cardiac reprogramming factors from embryonic fibroblast (Hashimoto et al. 2019). At this time, we observed a net decrease in the Gata4 network weights as observed by an increase in negative inflection points and a decrease in positive values. Mef2a showed a similar trend as Gata4 with a minor increase in network weights leading up to birth, which has been previously shown to be important in postnatal heart development and regulation (Desjardins

and Naya 2016). Srf shows a different trend with most of the weights being relatively low until P0 where there is a minor but noticeable uptick in the network weights. This observation matches the biological importance of Srf in early cardiac development and its critical role in maintaining adult heart function (Mokalled et al. 2015). Foxs1 demonstrates the most profound change over time with the initial weights being very low and increasing over time until embryonic day 16.5. After this time the weights begin to decrease into adulthood but never go away completely. This may be due to the role of Foxs1 as a key factor in vascular development (De Val 2011) which is important in earlier development.

Model Comparison

There have been a number of studies on mouse cardiac TF regulatory networks with one study looking at the regulatory networks of Gata4, Mef2a, Nkx2-5, and Srf and providing the interactions they identified (Schlesinger et al. 2011). We extracted the interactions of the aforementioned TFs from our network and compared it with the previously identified interactors (Figure 4A and S8). We found that our networks contain over 10,000 putative novel interactions (weight edge weight greater than 0) that were not reported previously. Interestingly, regardless of the timepoint, our networks captured only about 63% of Gata4 targets, 61.5% of Mef2a targets, and 57% Nkx2-5 and Srf targets of the previous network's interactions leaving a large portion of their networks unique to their analysis (Figure 4B). We speculate there are two likely explanations for the absence of a 100% overlap: 1) the previous network established interactions using the canonical distance-based cutoff leading to some genes being added or removed erroneously if cutoffs differed from our TAD boundaries or 2)

while our TAD boundaries are more accurate than distance-based cutoffs, the TADs we use are not fully representative of cardiac specific TADs leading to loss of some connections in our network. Regardless of the reason, we wanted to understand if the main overlap between our networks was due to the previous study finding the strongest interactors of the TFs. To test this, we performed the Kolmogorov–Smirnov test (KS-test) on the cumulative distribution between the overlap edge weights and the full edge weights (Figure 4C and Table S2). We found that in all time points the overlapping genes identified have higher mean edge weights than the total (Figure 4D). This implies that we are identifying true strongly interacting targets and a broad set of possible true but weakly interacting targets.

To further support the biological relevance of our networks, we selected the full TF network, the overlapping network, and the connections unique to our network, and performed GO-term enrichment analysis (Figure 5, S9). We see that in the case of Mef2a, there is a similar core of regulatory processes that are maintained from 10.5e and 8w (Figure 5). Of interest, we found that in younger 10.5e hearts there was significant enrichment for development related genes as opposed to older 8w hearts which had T cell activation terms enriched. This makes sense when considering recent studies showing Mef2a involvement in inflammation-related processes and the interaction of T cell activation and inflammation (Skapenko et al. 2005; Xiong et al. 2019). Furthermore, we found that overlapping targets between our data and previous data contained terms enriched for cardiac development while full and unique networks showed enrichment for cardiac related and general biological terms. Thus, it is reasonable to conclude that our network contains true biologically relevant interactions in cardiac tissue throughout development.

215 **Conclusions:**

216 Our results show that we were able to expand current methods for generating
 217 regulatory networks to take advantage of Topologically Associated Domains (TADs) to limit the
 218 predicted influence of enhancers. In this way, we were able to produce highly similar results as
 219 reported previously with the added benefit of the networks containing an expanded set of
 220 potentially relevant biological connections that can be explored. Additionally, we have
 221 developed a framework that can be exploited for a diverse array of species and cell types
 222 requiring only two experimental assays, H3K27ac ChIP-seq and RNA-seq. We believe this
 223 method opens the possibility for understanding deeper connections and new possibilities for
 224 biological discovery.

Materials and Methods

Data Preprocessing

TReNCo begins by generating distinct transcription start sites (TSSs), using a parsing tool, MakeGencodeTSS, for protein-coding genes from Gencode annotation files: Mouse Gencode version 4 by default. Promoters are then constructed using bedtools slot to extend 1000 nucleotides upstream and 200 nucleotides downstream of the TSS in a strand specific extension. Enhancer boundaries are then generated by using bedops merge to merge the user defined H3K27ac ChIP peak bed files and excluding overlaps with promoter regions.

A transcript expression matrix with normalized log2 TPM is generated from the provided RNA-seq expression tables with each row corresponding to a gene and the columns corresponding to a sample. The same is done for enhancers with bedtools coverage being used to calculate the coverage for each enhancer for each sample using the enhancer regions defined previously.

Log-Odds ratio for TF (TF) binding to promoters and enhancers is calculated with MEME-suite software, FIMO, using cis-bp motif database on promoter and enhancer sequences extracted from the genome, default mm10 (GRCm38), using bedtools getfasta and the bed files generated previously. TF matrices are constructed by reformatting the native output of FIMO and converting TF names to gene symbols

Model Algorithm and Matrix Factorization

We use the following annotations: M for a matrix, \mathbf{m}_j for a matrix column, and $m_{i,j}$ for a matrix element. TReNCo will, for each sample k , extract gene expression (\mathbf{g}^k) and enhancer expression (\mathbf{e}^k) vectors from the previously built gene (G) and enhancer (E) TPM matrices. From

the gene expression vector, TF genes will be extracted from the gene matrix (G) and scaled from 0 to 1 to form a network weighting score (G'^k).

Then for each TAD t genome interval defined in a bed file, default is provided for mice from a previous publication (Gittenberger-De Groot et al. 2005). Genes, promoters, and enhancers are isolated along with TF scores for promoters and enhancers. A distance weight matrix for the TAD (D^t) is built between all genes and enhancers and used to build a TAD specific enhancer by gene weight ($W^{k,t}$).

Calculate D^t distance weights using genomic positions ep_i and gp_j for the corresponding enhancer start peak position and gene start position.

$$y_{i,j} = \frac{1}{\log_2(|ep_i - gp_j|)}$$

$$y_{\max} = \max(Y), y_{\min} = \min(Y)$$

$$d_{i,j}^t = \frac{y_{i,j} - y_{\min}}{y_{\max} - y_{\min}}$$

Calculate $w_{i,j}^{k,t}$ enhancer by gene weight

$$w_{i,j}^{k,t} = d_{i,j}^t \sqrt{\log_2(e_{i,k} g_{j,k})}$$

TAD specific TF by enhancer score (M^t) and TF by gene score (P^t) are extracted from the FIMO matrices and scaled to the max value of the original matrices. We then factor the vectors and matrices, scale values with an arctan to condense higher values to a similar score approaching $\frac{\pi}{2}$. The resulting matrix is weighted by the network weighting score G'^k to form TAD specific TF gene regulatory network ($R^{k,t}$).

$$R^{k,t} = \frac{G'^k}{\max(G'^k)} \left(\arctan\left(\frac{P^t}{\max(P^t)} \times \mathbf{diag}(\mathbf{g}_k)\right) + \arctan\left(\frac{M^t}{\max(M^t)} \times W^{k,t}\right) \right)$$

267
 268 All $R^{k,t}$ matrices are concatenated together to form a full sample specific gene
 269 regulatory network R^k . Gene expression vectors for each sample are converted to gene symbols
 270 and output as node weights. The edge matrix is sparse and thus is converted to a vector with
 271 TF-gene links being preserved in a string key and zero values being removed. This edge vector is
 272 output to a file as the nodes are.

273 ENCODE Data

274 Gene expression and H3K27ac ChIP data was selected from ENCODE. Data was chosen
 275 using a script to select mouse heart data corresponding to embryonic day 10.5, 11.5, 12.5, 13.5,
 276 14.5, 15.5, 16.5, postnatal day 0 and 8 weeks old and had a matched set of Gene expression
 277 and ChIP-seq. In total every sample had at least technical duplicates with embryonic day 14.5,
 278 postnatal day 0, and 8 weeks old time points having 4 replicates of gene expression and
 279 embryonic day 14.5 having 4 ChIP-seq replicates. Two of the embryonic day 14.5 gene
 280 expression data were dropped due to poor correlation (average R^2 less than 0.7) with the rest
 281 of the data set. (Table S2 for accession numbers)

282 Statistical Analysis and Data Visualization

283 Plots and graphs were built using seaborn for python and ggplot2 for R scripts. All
 284 heatmaps were built in python and analyzed with scikit-learn. Gene networks from previous
 285 studies were downloaded from the corresponding journals and converted to a list. Genes
 286 networks for Gata4, Srf, Mef2a, and Nfx2-5 were subset from our networks and targets for
 287 these TFs were converted to lists. Venn-diagrams of gene list overlaps were built in python
 288 using venn2 package. Analysis of GO-terms was performed in R using enrichGO in clusterProfiler

289 with org.Mm.eg.db database. Plots for GO-terms were built using the built in dotplot function
290 in clusterProfiler.
291

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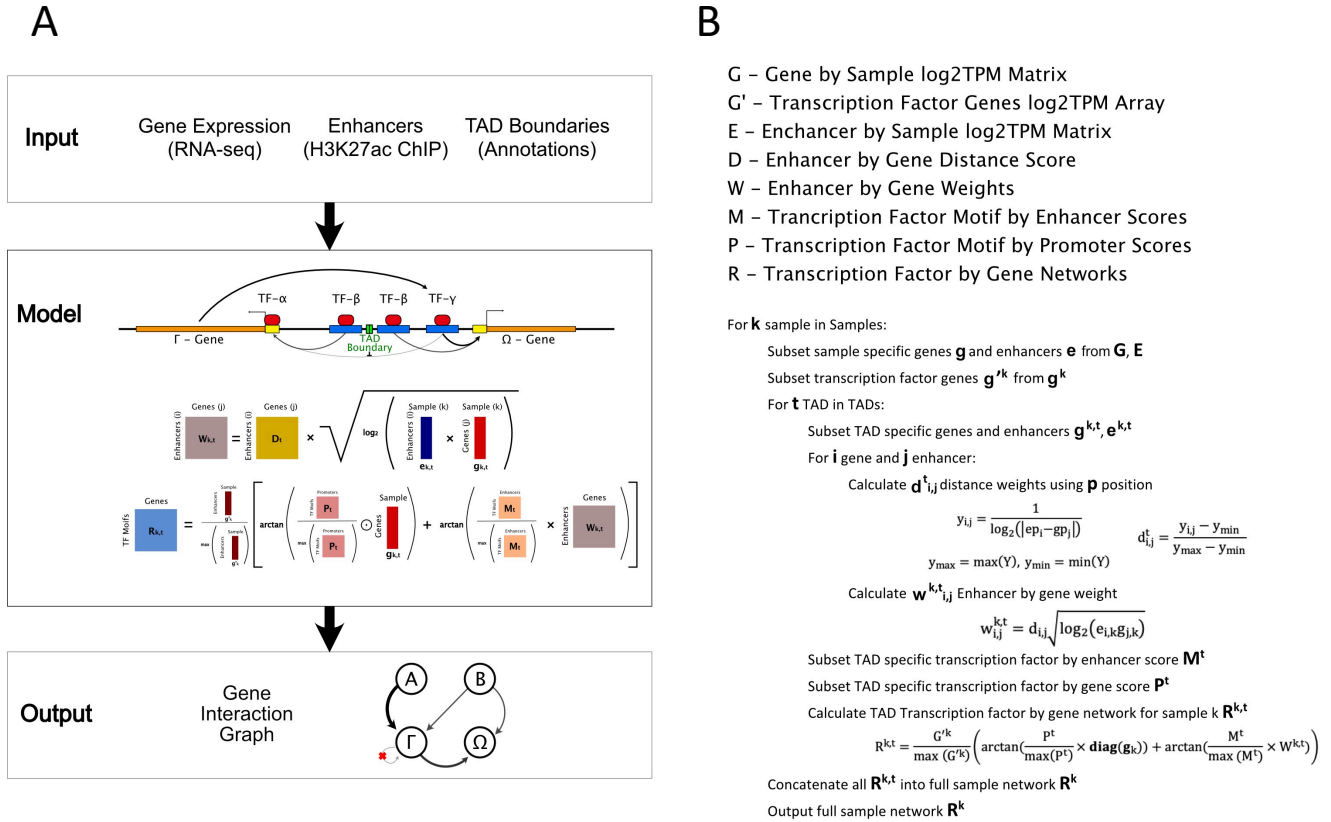
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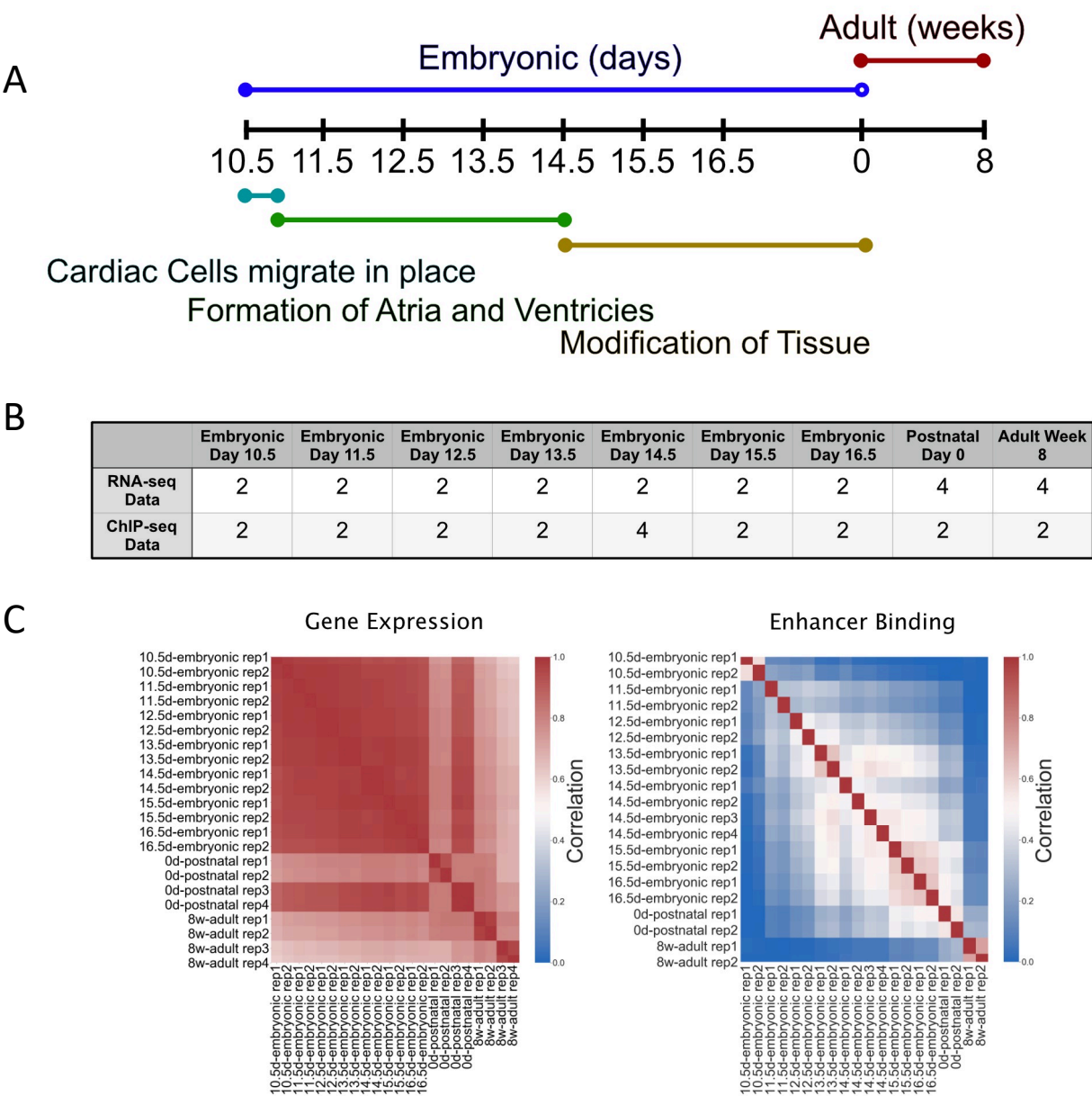
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Figure 1:



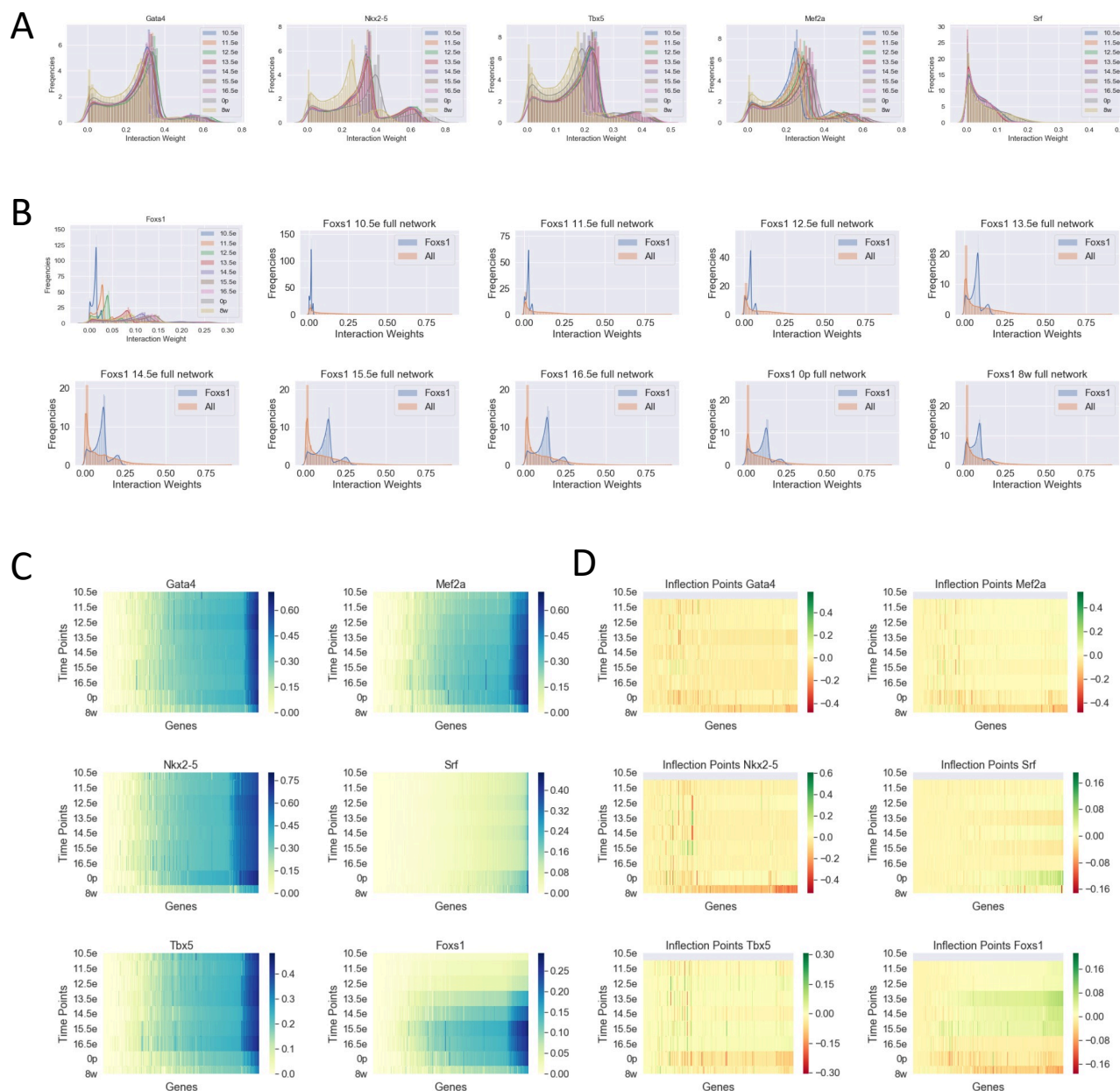
TReNCo model. A) Diagram with required inputs, gene expression, enhancers, and TAD boundaries input into a model using the basic equations shown leading to a gene interaction graph result. '+' and 'x' indicate standard matrix additions and multiplications, respectively. The other operations such as \odot , log, square root, and arctan are all element-wise. B) Pseudo-code for constructing a Gene Interaction Graph.

Figure 2:



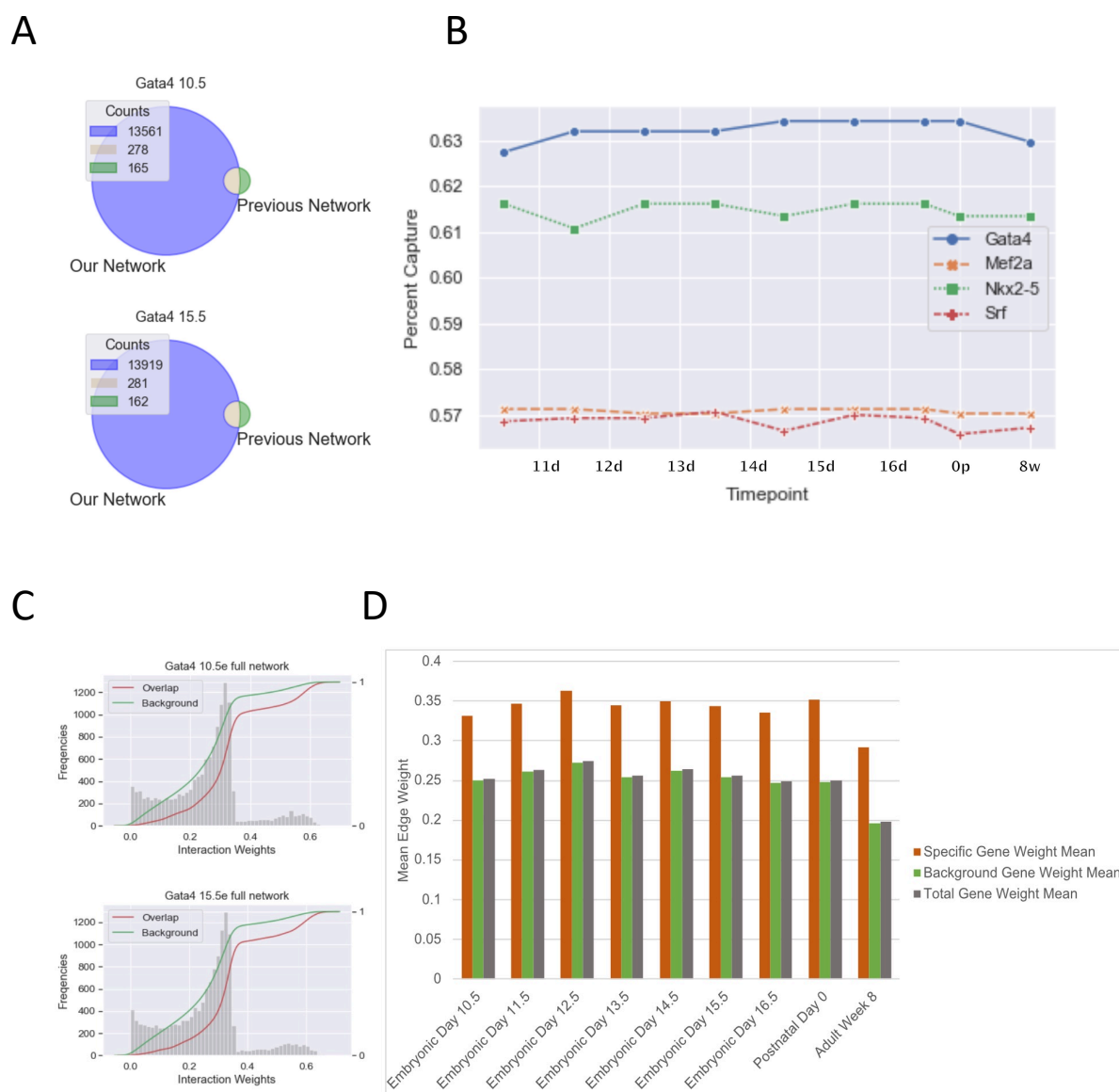
ENCODE Datasets. A) Timeline of basic mouse cardiovascular development with life stage on top and developmental stages on the bottom B) Number of samples for each timepoint and data type C) Correlation heatmap between samples and replicates at each time point

Figure 3:



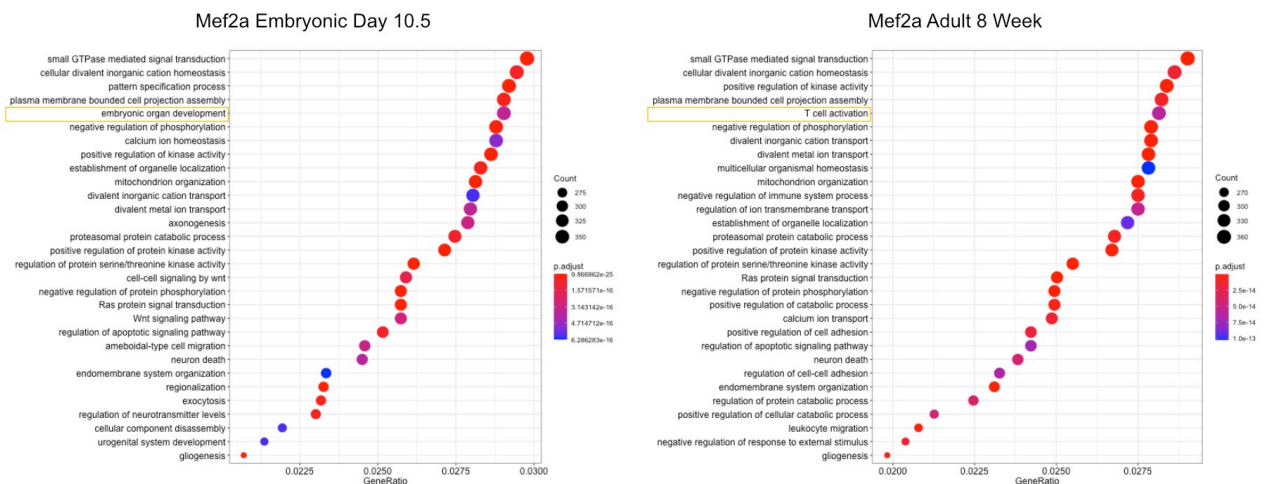
TF-Gene Edge Weights. A) Histograms of TF-gene interaction weights for 5 different genes, separated by developmental time points B) Histogram as in A, for Foxs1 TF separated into individual developmental time point plots C) Heatmap of TF-gene interaction weights sorted by time points D) Heatmap as in C, showing gene inflection points calculated by log2 the ratio of gene weights. Green indicates increase gene weight from the last time point while Red indicates a decrease.

Figure 4:



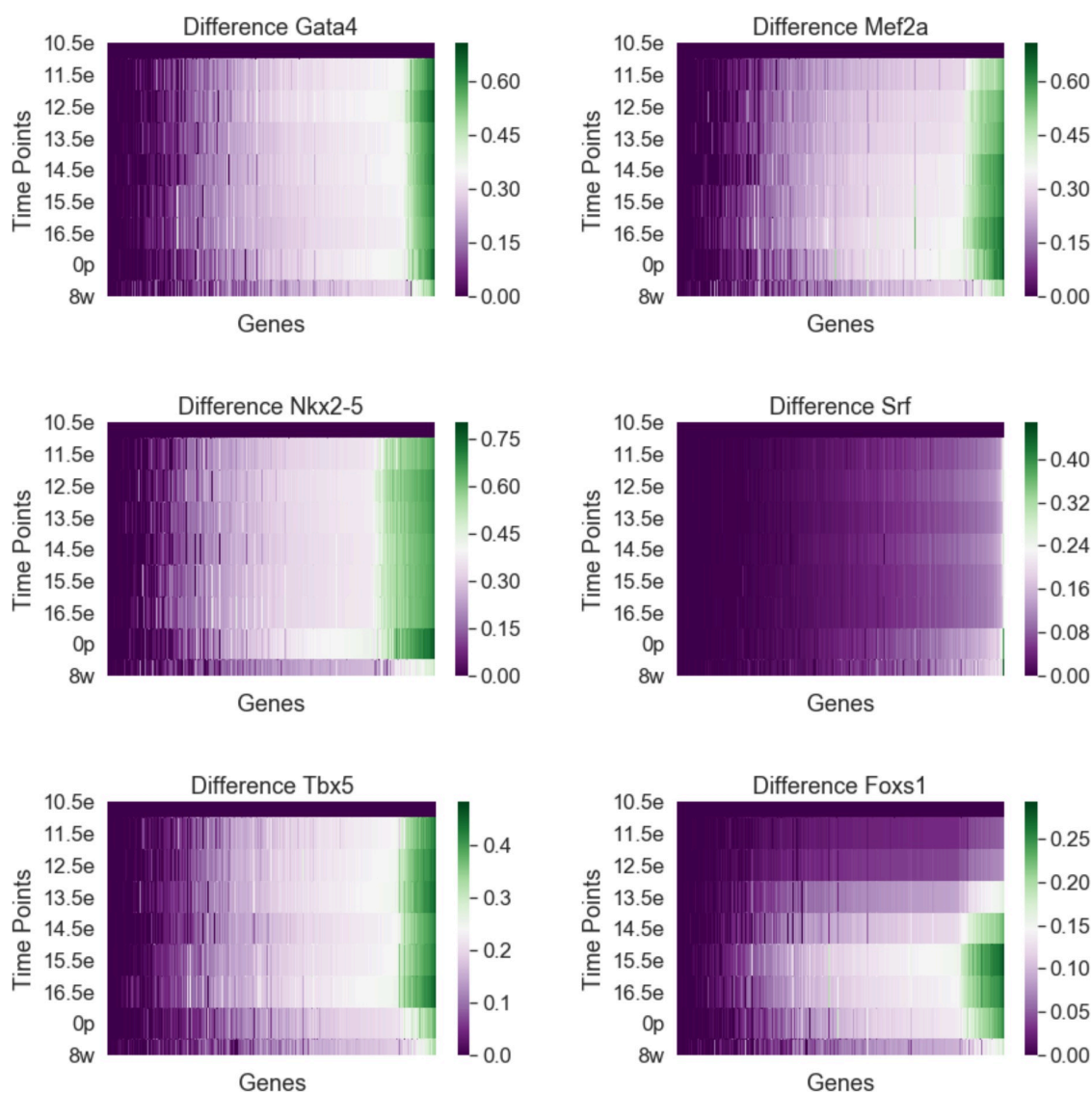
Model Comparison. A) TF-gene interaction Venn Diagram overlap between TReNCo model and previous study B) Line plot showing percent TF-gene interactions captured from a previous study with the TReNCo model C) CDF plot with weight of overlapping interactions vs background in TReNCo model D) Bar plot of mean TF-gene weights for Specific/overlapping interactions (red), all other background interactions (green) and all interactions (blue)

Figure 5:

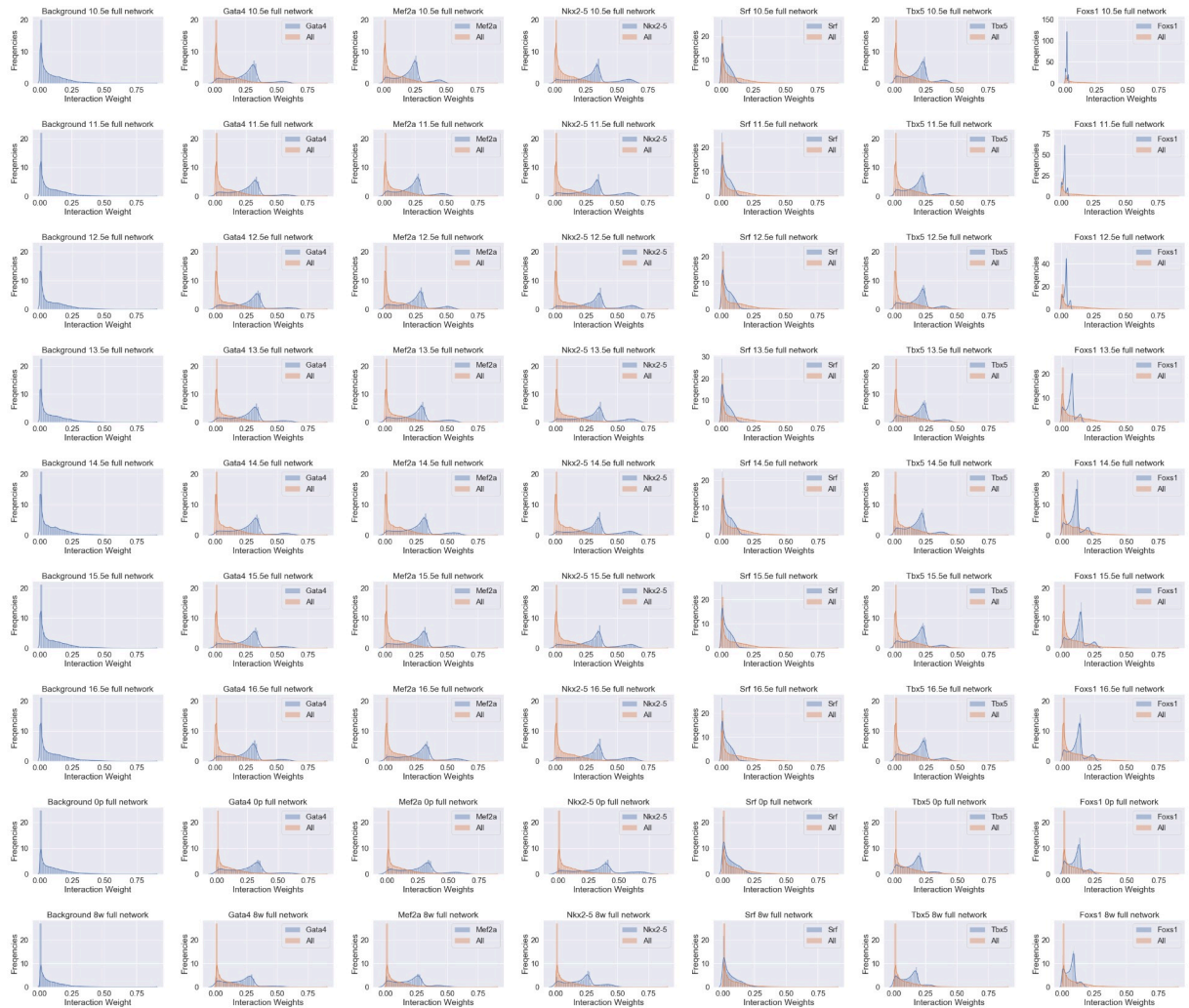


GO-term Enrichment. Enrichment for gene terms between embryonic day 10.5 and 8 week old adult heart. Highlighted terms show first difference in term list

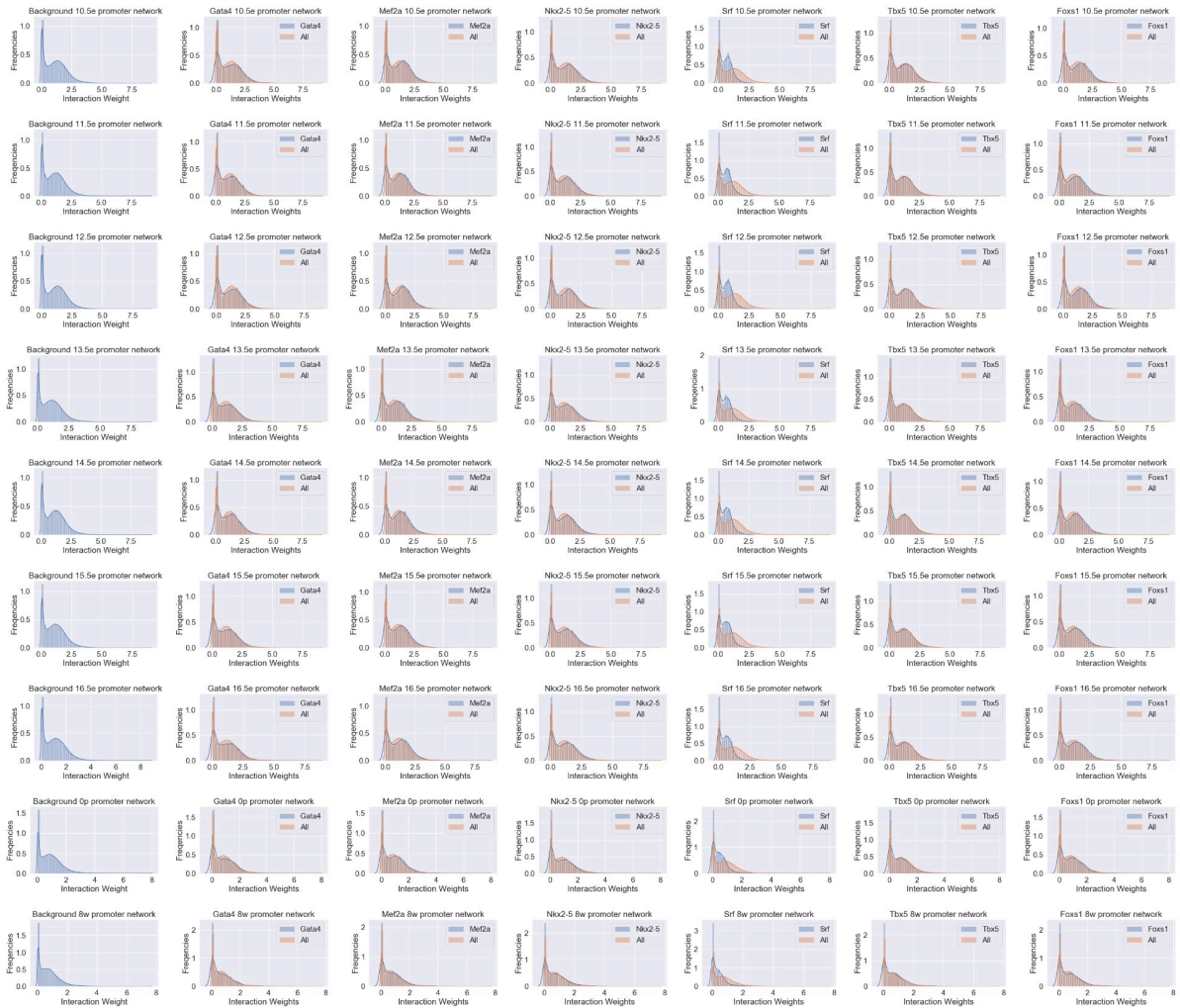
Supplement 1: Differential weights



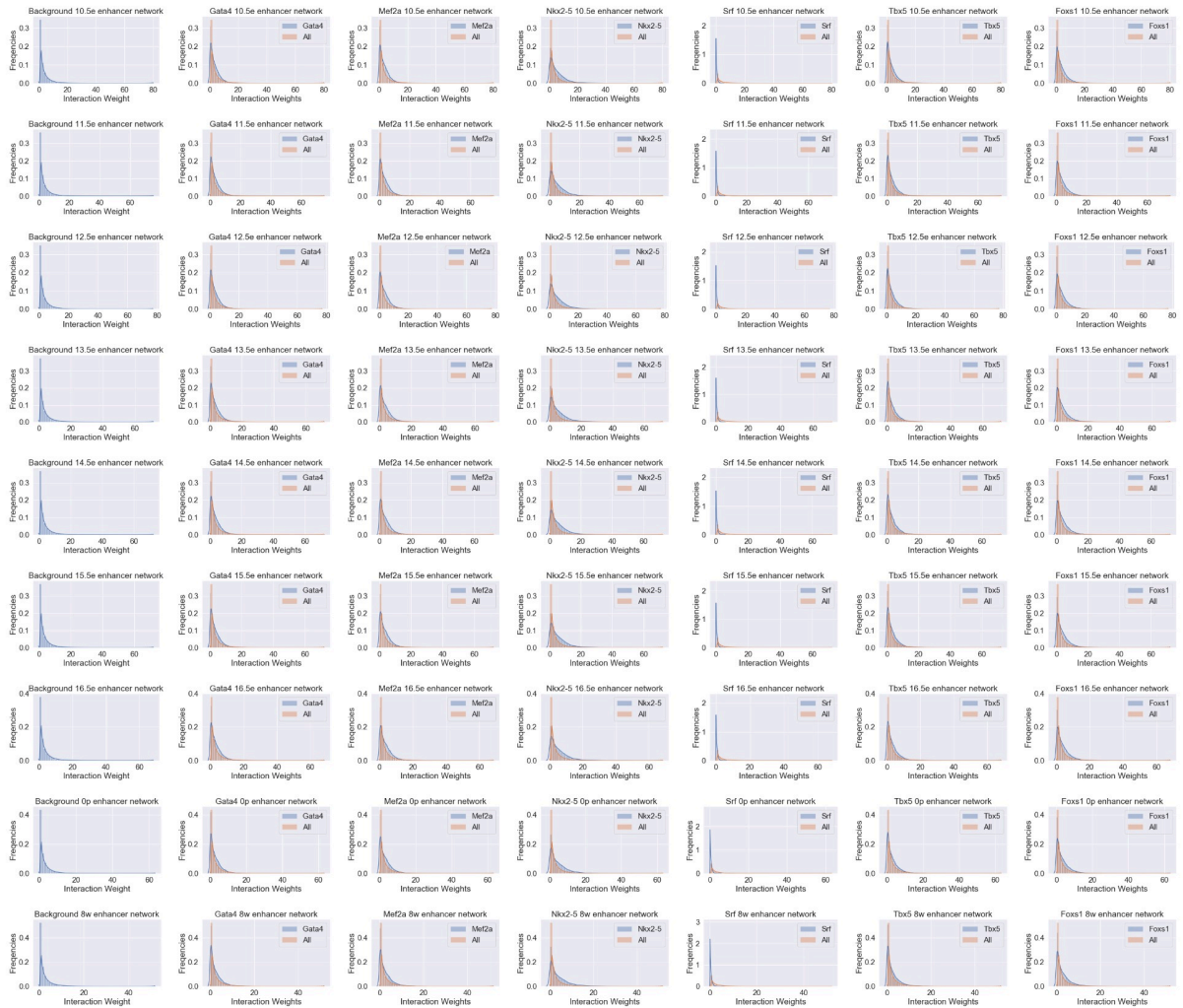
Supplement 2: All Distributions



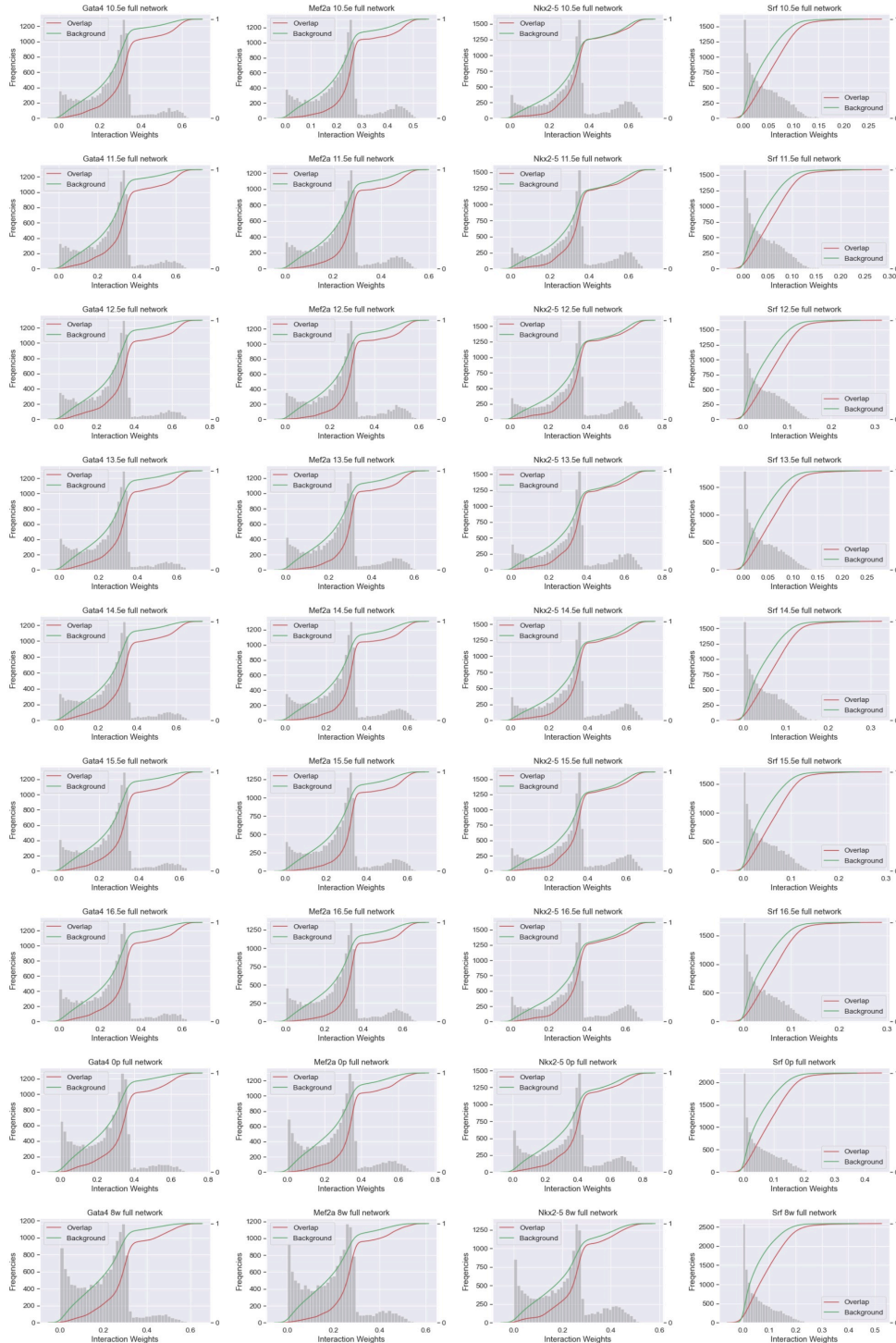
Supplement 3: Promoter Distributions



Supplement 4: Enhancer Distributions

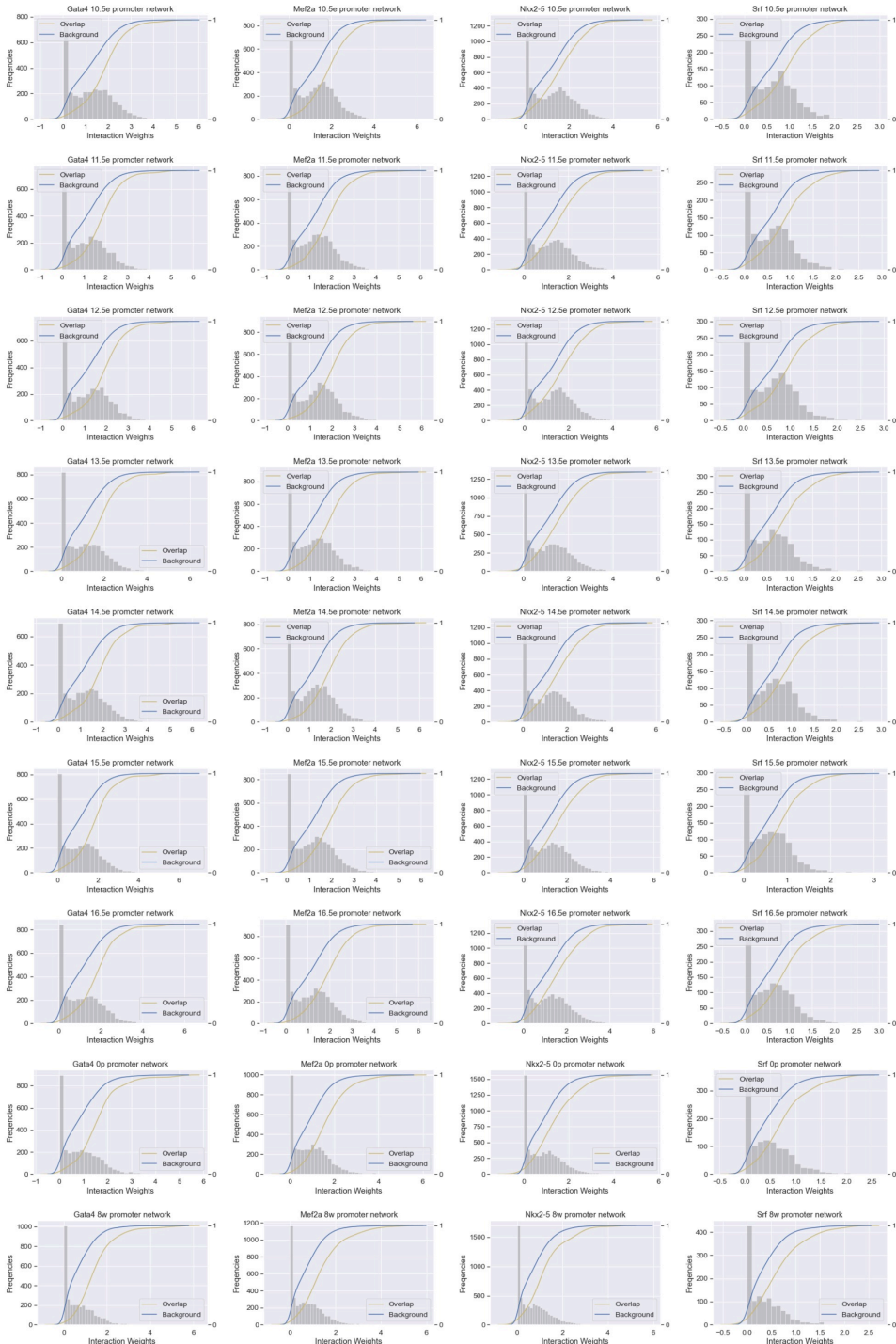


Supplemental 5: CDF Plots All Genes

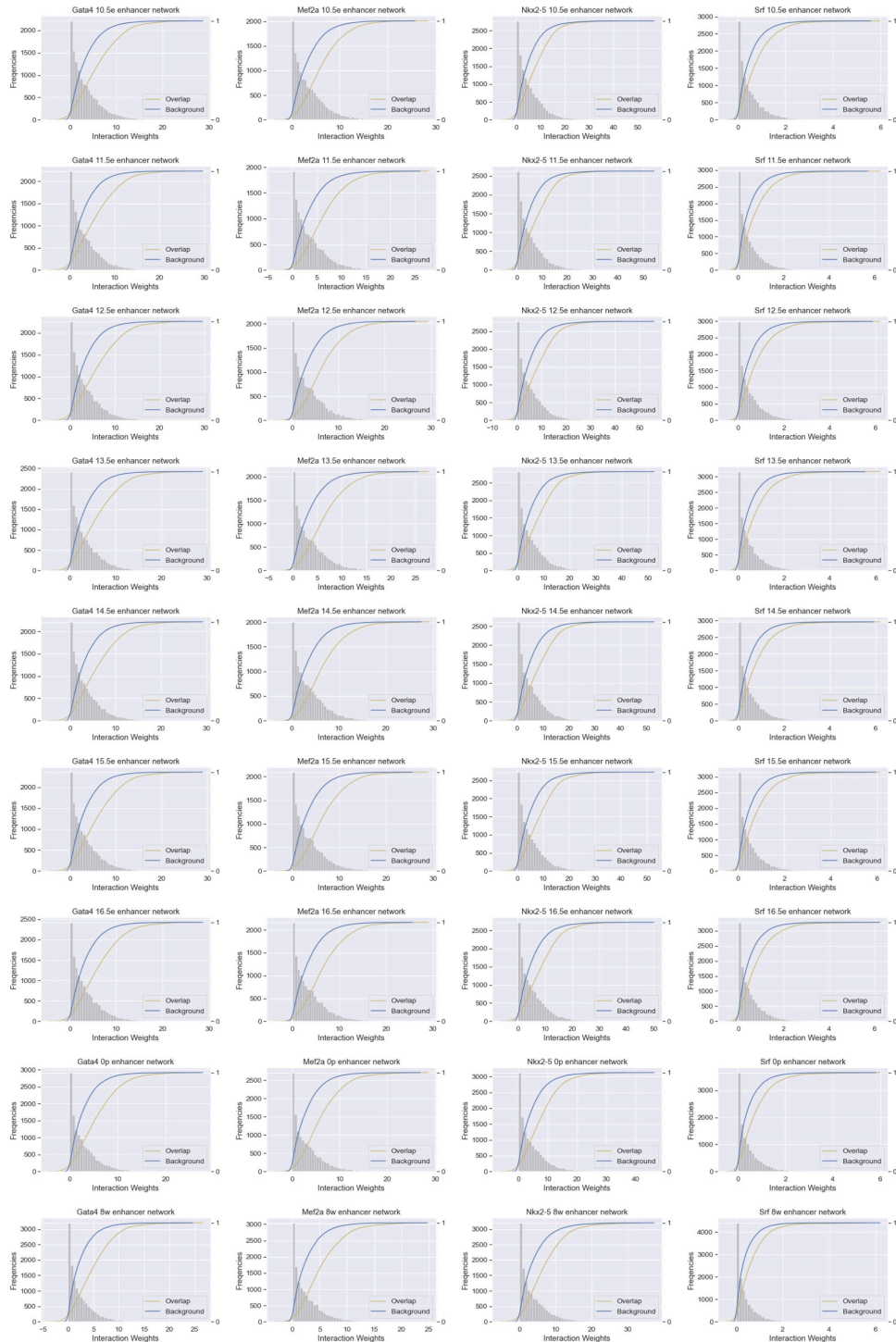


Supplement 6: CDF Plots

Promoters



Supplement 7: CDF Plots Enhancer



Supplement 8: All overlaps



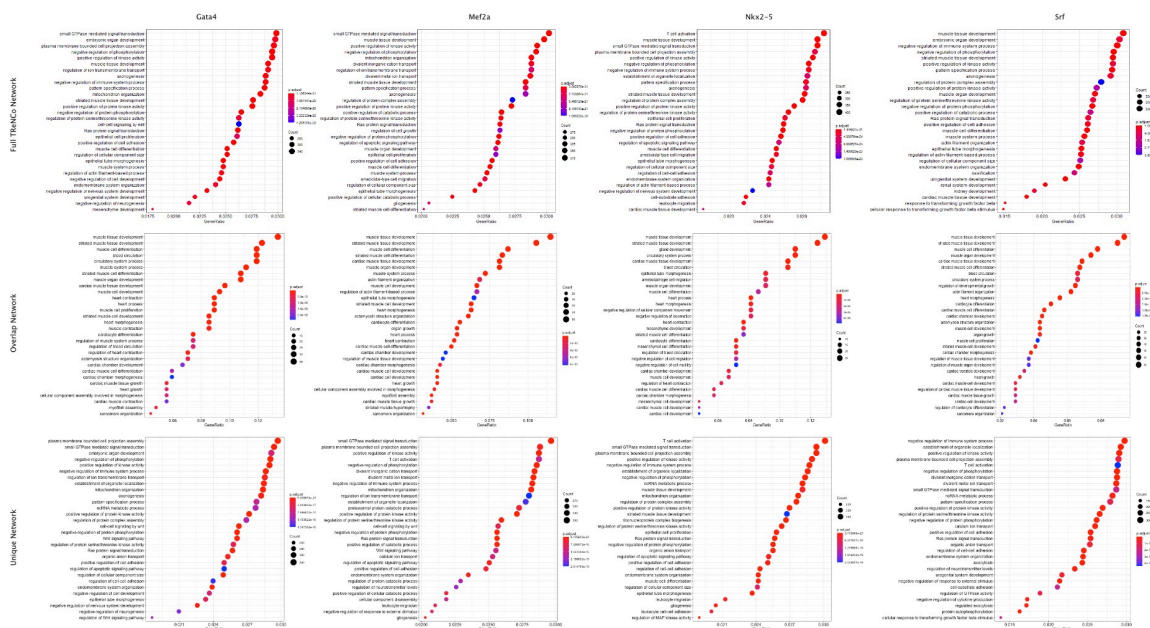


Table 1: Encode Data for validation

Time Point	RNA-seq Data				H3K27ac Data			
heart_10.5e	heart_10.5d-embryonic_rep1_RNA-seq_ENCF127FPD_ENCSR049	heart_10.5d-embryonic_rep2_RNA-seq_ENCF721XZC_ENCSR049			heart_10.5d-embryonic_rep1_H3K27ac_EN	heart_10.5d-embryonic_rep2_H3K27ac_EN		
	UUU	UUU			CFB847FKM_ENCSR5825PN	CF6601EJB_ENCSR5825PN		
heart_11.5e	heart_11.5d-embryonic_rep1_RNA-seq_ENCF226IWR_ENCSR691	heart_11.5d-embryonic_rep2_RNA-seq_ENCF540EIL_ENCSR6910			heart_11.5d-embryonic_rep1_H3K27ac_EN	heart_11.5d-embryonic_rep2_H3K27ac_EN		
	OPQ	PQ			CF6635BLS_ENCSR222IHX	CF4722SL_ENCSR222IHX		
heart_12.5e	heart_12.5d-embryonic_rep1_RNA-seq_ENCF125TAY_ENCSR150	heart_12.5d-embryonic_rep2_RNA-seq_ENCF824DCQ_ENCSR150			heart_12.5d-embryonic_rep1_H3K27ac_EN	heart_12.5d-embryonic_rep2_H3K27ac_EN		
	CUE	CUE			CF218WWW_ENCSR123MLY	CF778SLY_ENCSR123MLY		
heart_13.5e	heart_13.5d-embryonic_rep1_RNA-seq_ENCF242GMD_ENCSR28	heart_13.5d-embryonic_rep2_RNA-seq_ENCF976CYB_ENCSR284			heart_13.5d-embryonic_rep1_H3K27ac_EN	heart_13.5d-embryonic_rep2_H3K27ac_EN		
	4YKY	YKY			CF982HXU_ENCSR699XHY	CF695YAS_ENCSR699XHY		
heart_14.5e	heart_14.5d-embryonic_rep1_RNA-seq_ENCF111JGW_ENCSR727	heart_14.5d-embryonic_rep1_RNA-seq_ENCF662WLV_ENCSR000	heart_14.5d-embryonic_rep2_RNA-seq_ENCF540BJT_ENCSR727	heart_14.5d-embryonic_rep2_RNA-seq_ENCF705YYN_ENCSR000	heart_14.5d-embryonic_rep1_H3K27ac_EN	heart_14.5d-embryonic_rep1_H3K27ac_EN	heart_14.5d-embryonic_rep2_H3K27ac_EN	heart_14.5d-embryonic_rep2_H3K27ac_EN
	FHP	CHF	HP	CHF	CF098HOY_ENCSR360ANE	CF176BPZ_ENCSR000CDK	CF3100LKR_ENCSR000CDK	CF5549PWG_ENCSR360ANE
heart_15.5e	heart_15.5d-embryonic_rep1_RNA-seq_ENCF440PW8_ENCSR597	heart_15.5d-embryonic_rep2_RNA-seq_ENCF219PVC_ENCSR597			heart_15.5d-embryonic_rep1_H3K27ac_EN	heart_15.5d-embryonic_rep2_H3K27ac_EN		
	UZW	UZW			CF576RBC_ENCSR574VME	CF261KJB_ENCSR574VME		
heart_16.5e	heart_16.5d-embryonic_rep1_RNA-seq_ENCF415JBL_ENCSR020D	heart_16.5d-embryonic_rep2_RNA-seq_ENCF871JIGQ_ENCSR020			heart_16.5d-embryonic_rep1_H3K27ac_EN	heart_16.5d-embryonic_rep2_H3K27ac_EN		
	GG	DGG			CF7716BZP_ENCSR846PJO	CF095YRX_ENCSR846PJO		
heart_0p	heart_0d-postnatal_rep1_RNA-seq_ENCF799NMY_ENCSR035	heart_0d-postnatal_rep1_RNA-seq_ENCF817KPY_ENCSR5265	heart_0d-postnatal_rep2_RNA-seq_ENCF155GNG_ENCSR526	heart_0d-postnatal_rep2_RNA-seq_ENCF259NEX_ENCSR035	heart_0d-postnatal_rep1_H3K27ac_EN	heart_0d-postnatal_rep2_H3K27ac_EN		
	DIJ	EX	SEX	DIJ	FF441FKM_ENCSR675HDX	FF659KMV_ENCSR675HDX		
heart_8w_adult	heart_8w-adult_rep1_RNA-seq_ENCF929JPM_ENCSR000	heart_8w-adult_rep1_RNA-seq_ENCF948VMD_ENCSR000	heart_8w-adult_rep2_RNA-seq_ENCF204IFN_ENCSR000B	heart_8w-adult_rep2_RNA-seq_ENCF6537ZM_ENCSR000	heart_8w-adult_rep1_H3K27ac_EN	heart_8w-adult_rep2_H3K27ac_EN		
	BYQ	CGZ	YQ	CGZ	3VMQ_ENCSR000CDF	2VUL_ENCSR000CDF		

Table 2: KS-test for all CDFs

	Specific Gene Weight Mean	Background Gene Weight Mean	Total Gene Weight Mean	KS test results
process/heart_10.5e_if_gene_full_matrix.txt	0.3317	0.2503	0.2919	KStest_Ks_2sampleResult(statistic=0.30708121250014, pvalue=7.4034254803055e-23)
Gene:Gat4				
Gene:Me2a	0.2734	0.2084	0.2111	KStest_Ks_2sampleResult(statistic=0.303210893879151, pvalue=6.557259117991856e-45)
Gene:Nkx2-5	0.3571	0.311	0.3117	KStest_Ks_2sampleResult(statistic=0.191252354390591, pvalue=1.4795201259462122e-07)
Gene:Srf	0.0591	0.0406	0.0418	KStest_Ks_2sampleResult(statistic=0.217426815875645, pvalue=2.004081339291428e-31)
process/heart_11.5e_if_gene_full_matrix.txt				
Gene:Gat4	0.3464	0.2613	0.2863	KStest_Ks_2sampleResult(statistic=0.3122944501516235, pvalue=1.177901665158080e-23)
Gene:Me2a	0.2929	0.2224	0.2253	KStest_Ks_2sampleResult(statistic=0.306596041158062, pvalue=7.0398043972548e-46)
Gene:Nkx2-5	0.3612	0.3094	0.3302	KStest_Ks_2sampleResult(statistic=0.21612900810325442, pvalue=1.884927084862188e-09)
Gene:Srf	0.0602	0.041	0.0423	KStest_Ks_2sampleResult(statistic=0.2173430404076017, pvalue=2.26295808946739e-31)
process/heart_12.5e_if_gene_full_matrix.txt				
Gene:Gat4	0.3626	0.2724	0.2742	KStest_Ks_2sampleResult(statistic=0.3104851931904231, pvalue=2.1514393932085e-23)
Gene:Me2a	0.3177	0.2398	0.2443	KStest_Ks_2sampleResult(statistic=0.31487918462506475, pvalue=2.07025105078401e-40)
Gene:Nkx2-5	0.3777	0.3242	0.325	KStest_Ks_2sampleResult(statistic=0.22039920410206423, pvalue=6.7886093970041e-10)
Gene:Srf	0.0683	0.0464	0.0478	KStest_Ks_2sampleResult(statistic=0.222089700769176, pvalue=8.6091174891037e-33)
process/heart_13.5e_if_gene_full_matrix.txt				
Gene:Gat4	0.3446	0.2546	0.2564	KStest_Ks_2sampleResult(statistic=0.3161653587660625, pvalue=2.993276796954617e-34)
Gene:Me2a	0.3251	0.2418	0.2452	KStest_Ks_2sampleResult(statistic=0.3265280666861485, pvalue=4.10559183108038e-31)
Gene:Nkx2-5	0.3737	0.3159	0.3168	KStest_Ks_2sampleResult(statistic=0.2328631227612959, pvalue=5.34183893649418e-11)
Gene:Srf	0.0596	0.0401	0.0413	KStest_Ks_2sampleResult(statistic=0.22378402572348355, pvalue=2.1252824180542e-33)
process/heart_14.5e_if_gene_full_matrix.txt				
Gene:Gat4	0.35	0.2625	0.2643	KStest_Ks_2sampleResult(statistic=0.308914084958923, pvalue=3.1016413918002e-23)
Gene:Me2a	0.3483	0.2625	0.2661	KStest_Ks_2sampleResult(statistic=0.321602781450757, pvalue=1.9658774799912377e-50)
Gene:Nkx2-5	0.3704	0.3151	0.316	KStest_Ks_2sampleResult(statistic=0.2251651643458385, pvalue=2.64765370022969e-30)
Gene:Srf	0.0699	0.0475	0.049	KStest_Ks_2sampleResult(statistic=0.2228997388782365, pvalue=7.5709626852415e-33)
process/heart_15.5e_if_gene_full_matrix.txt				
Gene:Gat4	0.3453	0.254	0.2558	KStest_Ks_2sampleResult(statistic=0.33150082172171014, pvalue=8.67872441738656e-27)
Gene:Me2a	0.3471	0.2586	0.2622	KStest_Ks_2sampleResult(statistic=0.332731130802745, pvalue=4.76395183770398e-34)
Gene:Nkx2-5	0.3725	0.3148	0.3157	KStest_Ks_2sampleResult(statistic=0.235252170752379, pvalue=3.2160333830638e-11)
Gene:Srf	0.0628	0.0421	0.0434	KStest_Ks_2sampleResult(statistic=0.2354992051246475, pvalue=6.24030637128039e-37)
process/heart_16.5e_if_gene_full_matrix.txt				
Gene:Gat4	0.3354	0.2474	0.2491	KStest_Ks_2sampleResult(statistic=0.318404607073955, pvalue=2.90444838925187e-28)
Gene:Me2a	0.3629	0.2691	0.2728	KStest_Ks_2sampleResult(statistic=0.33504088163195184, pvalue=8.05750616754812e-55)
Gene:Nkx2-5	0.3721	0.3143	0.3152	KStest_Ks_2sampleResult(statistic=0.23732472904248347, pvalue=2.064800461239954e-11)
Gene:Srf	0.0623	0.0417	0.043	KStest_Ks_2sampleResult(statistic=0.2356818991735046, pvalue=5.86604273797058e-37)
process/heart_17.5e_if_gene_full_matrix.txt				
Gene:Gat4	0.3515	0.2479	0.2499	KStest_Ks_2sampleResult(statistic=0.350132463905747, pvalue=9.628119830019224e-30)
Gene:Me2a	0.3616	0.2562	0.2605	KStest_Ks_2sampleResult(statistic=0.3476302128856224, pvalue=8.107620704135622e-59)
Gene:Nkx2-5	0.4125	0.3327	0.3319	KStest_Ks_2sampleResult(statistic=0.2556762700846173, pvalue=4.08097687239962e-13)
Gene:Srf	0.0922	0.059	0.0612	KStest_Ks_2sampleResult(statistic=0.244811016310683, pvalue=3.9283511723992e-39)
process/heart_18.5e_if_gene_full_matrix.txt				
Gene:Gat4	0.3918	0.1963	0.1982	KStest_Ks_2sampleResult(statistic=0.3651465430046206, pvalue=8.37444608295591e-33)
Gene:Me2a	0.2773	0.1903	0.1903	KStest_Ks_2sampleResult(statistic=0.3497158217181099, pvalue=1.3691126693409823e-59)
Gene:Nkx2-5	0.2622	0.2045	0.2054	KStest_Ks_2sampleResult(statistic=0.2675232621695956, pvalue=2.521697499028624e-14)
Gene:Srf	0.096	0.0599	0.0627	KStest_Ks_2sampleResult(statistic=0.2396483193938245, pvalue=6.85798496651153e-38)
process/heart_19.5e_if_gene_full_matrix.txt				
Gene:Gat4	0.60041	3.31	3.3643	KStest_Ks_2sampleResult(statistic=0.3103460096360705, pvalue=4.582034303246030e-22)
Gene:Me2a	6.2362	3.3753	3.4659	KStest_Ks_2sampleResult(statistic=0.352074874934672, pvalue=1.821315988496026e-55)
Gene:Nkx2-5	8.1453	5.2617	5.4651	KStest_Ks_2sampleResult(statistic=0.241209533100712, pvalue=1.264584056471242e-30)
Gene:Srf	0.7983	0.4981	0.5172	KStest_Ks_2sampleResult(statistic=0.21349571888770952, pvalue=3.046864004944067e-29)
process/heart_11.5e_if_gene_enhancer_matrix.txt				
Gene:Gat4	0.6043	3.2838	3.2408	KStest_Ks_2sampleResult(statistic=0.3173570154377778, pvalue=3.26819538911193e-31)
Gene:Me2a	6.4179	3.3792	3.5062	KStest_Ks_2sampleResult(statistic=0.360991853005344, pvalue=2.97991385103751e-58)
Gene:Nkx2-5	8.3	5.212	5.2607	KStest_Ks_2sampleResult(statistic=0.258778570200234, pvalue=4.223990160091255e-12)
Gene:Srf	0.7908	0.4914	0.5114	KStest_Ks_2sampleResult(statistic=0.2126494555400794, pvalue=4.685624602248737e-29)
process/heart_12.5e_if_gene_enhancer_matrix.txt				
Gene:Gat4	6.2722	3.3678	3.4272	KStest_Ks_2sampleResult(statistic=0.3214057796881, pvalue=9.123661778902185e-24)
Gene:Me2a	6.7297	3.4771	3.6105	KStest_Ks_2sampleResult(statistic=0.376690544948463, pvalue=5.78546035773634e-63)
Gene:Nkx2-5	8.5965	5.4334	5.4334	KStest_Ks_2sampleResult(statistic=0.363889397394114, pvalue=1.248173306564099e-12)
Gene:Srf	0.8201	0.5058	0.5205	KStest_Ks_2sampleResult(statistic=0.2173738770084626, pvalue=5.02380572139059e-30)
process/heart_13.5e_if_gene_enhancer_matrix.txt				
Gene:Gat4	6.0889	3.2119	3.2697	KStest_Ks_2sampleResult(statistic=0.3265193151067715, pvalue=1.436104877583042e-24)
Gene:Me2a	6.6017	3.3445	3.4758	KStest_Ks_2sampleResult(statistic=0.3861588603099518, pvalue=1.27040499123424e-48)
Gene:Nkx2-5	8.3325	5.1068	5.1566	KStest_Ks_2sampleResult(statistic=0.276617150612356, pvalue=7.69866826493522e-14)
Gene:Srf	0.7992	0.4871	0.5074	KStest_Ks_2sampleResult(statistic=0.2196719580836925, pvalue=4.52724513140699e-31)
process/heart_14.5e_if_gene_enhancer_matrix.txt				
Gene:Gat4	6.1175	3.2669	3.3261	KStest_Ks_2sampleResult(statistic=0.328744543226156, pvalue=2.15051462529931e-31)
Gene:Me2a	6.6969	3.3755	3.3755	KStest_Ks_2sampleResult(statistic=0.3745033368611531, pvalue=1.785828283979e-62)
Gene:Nkx2-5	8.4931	5.1887	5.2409	KStest_Ks_2sampleResult(statistic=0.2816470319319122, pvalue=2.932727397270516e-14)
Gene:Srf	0.8084	0.4985	0.5191	KStest_Ks_2sampleResult(statistic=0.2168666831895187, pvalue=5.109790183744581e-30)
process/heart_15.5e_if_gene_enhancer_matrix.txt				
Gene:Gat4	6.1475	3.2552	3.2892	KStest_Ks_2sampleResult(statistic=0.33913943187454043, pvalue=1.440789302022382e-26)
Gene:Me2a	6.735	3.3952	3.5284	KStest_Ks_2sampleResult(statistic=0.374995942140961, pvalue=1.549785425993367e-61)
Gene:Nkx2-5	8.5237	5.1811	5.2418	KStest_Ks_2sampleResult(statistic=0.28724629464461, pvalue=2.6576380094826e-15)
Gene:Srf	0.8133	0.4936	0.5142	KStest_Ks_2sampleResult(statistic=0.2284825121058712, pvalue=1.136939958184256e-33)
process/heart_16.5e_if_gene_enhancer_matrix.txt				
Gene:Gat4	6.173	3.2343	3.2826	KStest_Ks_2sampleResult(statistic=0.3456747573511122, pvalue=2.823072086307466e-27)
Gene:Me2a	6.7717	3.3942	3.5279	KStest_Ks_2sampleResult(statistic=0.3879630784085931, pvalue=2.38327950854412e-47)
Gene:Nkx2-5	8.5281	5.1679	5.2187	KStest_Ks_2sampleResult(statistic=0.29148349642179056, pvalue=2.518547013487967e-17)
Gene:Srf	0.8134	0.4937	0.5141	KStest_Ks_2sampleResult(statistic=0.22623684876665334, pvalue=6.23650189129188e-33)
process/heart_17.5e_if_gene_enhancer_matrix.txt				
Gene:Gat4	5.6621	2.8062	2.8641	KStest_Ks_2sampleResult(statistic=0.3513911897838063, pvalue=8.42565300279567e-29)
Gene:Me2a	6.2074	2.9601	3.0817	KStest_Ks_2sampleResult(statistic=0.3954938250033885, pvalue=8.9521896708385e-70)
Gene:Nkx2-5	7.8701	4.5188	4.5704	KStest_Ks_2sampleResult(statistic=0.307638026419781, pvalue=9.7842513448221e-17)
Gene:Srf	0.7423	0.4322	0.4523	KStest_Ks_2sampleResult(statistic=0.23681741493117307, pvalue=9.86362193999152e-36)
process/heart_18.5e_if_gene_enhancer_matrix.txt				
Gene:Gat4	4.9757	2.3587	2.41	KStest_Ks_2sampleResult(statistic=0.3648311988868314, pvalue=4.679516820907115e-31)
Gene:Me2a	5.5333	2.5017	2.6444	KStest_Ks_2sampleResult(statistic=0.3936530038079007, pvalue=3.341241394248354e-66)
Gene:Nkx2-5	6.8338	3.8547	3.8995	KStest_Ks_2sampleResult(statistic=0.328960632394376, pvalue=1.63466161498441e-24)
Gene:Srf	0.6411	0.3689	0.3861	KStest_Ks_2sampleResult(statistic=0.23206214028577817, pvalue=1.18931572860464e-34)
process/heart_10.5e_if_gene_promoter_matrix.txt				
Gene:Gat4	1.8245	1.1351	1.1528	KStest_Ks_2sampleResult(statistic=0.356466842626196, pvalue=1.429488026604959e-09)
Gene:Me2a	1.8732	1.1759	1.2066	KStest_Ks_2sampleResult(statistic=0.3218657382797175, pvalue=3.10824468054381e-15)
Gene:Nkx2-5	1.5991	1.1685	1.1743	KStest_Ks_2sampleResult(statistic=0.2104318508023106, pvalue=0.0011660993130366837)
Gene:Srf	0.9207	0.5837	0.6087	KStest_Ks_2sampleResult(statistic=0.289840596668005, pvalue=1.4964222105049977e-06)
process/heart_11.5e_if_gene_promoter_matrix.txt				
Gene:Gat4	1.804	1.0846	1.1036	KStest_Ks_2sampleResult(statistic=0.3846583205683353, pvalue=7.15395520007367e-11)
Gene:Me2a	1.8349	1.1221	1.154	KStest_Ks_2sampleResult(statistic=0.333053117433583, pvalue=2.9976021664870272e-15)
Gene:Nkx2-5	1.6079	1.1091	1.1158	KStest_Ks_2sampleResult(statistic=0.234712314485458, pvalue=0.0002438794801404336)
Gene:Srf	0.8831	0.5616	0.5859	KStest_Ks_2sampleResult(statistic=0.3105287569573284, pvalue=1.86364730981856e-07)
process/heart_12.5e_if_gene_promoter_matrix.txt				
Gene:Gat4	1.9088	1.1345	1.1547	KStest_Ks_2sampleResult(statistic=0.3829986131191435, pvalue=8.79513494389751e-11)
Gene:Me2a	1.9473	1.1164	1.1383	KStest_Ks_2sampleResult(statistic=0.374995942140961, pvalue=4.4468920802626e-15)
Gene:Nkx2-5	1.9095	1.1607	1.1679	KStest_Ks_2sampleResult(statistic=0.23118460168137174, pvalue=0.002480241403118679)
Gene:Srf	0.9121	0.5845	0.6092	KStest_Ks_2sampleResult(statistic=0.317620560389717, pvalue=1.466104646827902e-09)
process/heart_13.5e_if_gene_promoter_matrix.txt				
Gene:Gat4	1.7948	1.0429	1.0818	KStest_Ks_2sampleResult(statistic=0.38117882809970887, pvalue=7.997391637815099e-11)
Gene:Me2a	1.8563	1.0966	1.1296	KStest_Ks_2sampleResult(statistic=0.3666666666666644, pvalue=4.298697960381105e-15)
Gene:Nkx2-5	1.5839	1.0896	1.0963	KStest_Ks_2sampleResult(statistic=0.2094010561338677, pvalue=0.00023392393242991)
Gene:Srf	0.8735	0.5458	0.5697	KStest_Ks_2sampleResult(statistic=0.28768726622215, pvalue=1.815356607814955e-26)
process/heart_14.5e_if_gene_promoter_matrix.txt				
Gene:Gat4	1.8564	1.1011	1.1212	KStest_Ks_2sampleResult(statistic=0.4028869149299624, pvalue=6.4509508848447e-12)
Gene:Me2a	1.9134	1.1383	1.1729	KStest_Ks_2sampleResult(statistic=0.35663611250627825, pvalue=2.88657986405407e-15)
Gene:Nkx2-5	1.6401	1.1262	1.1332	KStest_Ks_2sampleResult(statistic=0.2369931654108887, pvalue=0.000291207296253063)
Gene:Srf	0.8825	0.5731	0.5968	KStest_Ks_2sampleResult(statistic=0.291633984377838, pvalue=1.103778129583554e-06)
process/heart_15.5e_if_gene_promoter_matrix.txt				
Gene:Gat4	1.8261	1.0579	1.077	KStest_Ks_2sampleResult(statistic=0.407581982373154, pvalue=6.52622405645098e-12)
Gene:Me2a	1.9071	1.1098	1.1442	KStest_Ks_2sampleResult(statistic=0.3665791838440385, pvalue=3.231468511126287e-15)
Gene:Nkx2-5	1.6302	1.1056	1.1125	KStest_Ks_2sampleResult(statistic=0.24486709077439958, pvalue=8.2618207573740e-05)
Gene:Srf	0.8819	0.5601	0.5837	KStest_Ks_2sampleResult(statistic=0.318895719316471, pvalue=5.99435978256038e-08)
process/heart_16.5e_if_gene_promoter_matrix.txt				
Gene:Gat4	1.8356	1.0536	1.0727	KStest_Ks_2sampleResult(statistic=0.4001325508079199, pvalue=8.601206156385e-12)
Gene:Me2a	1.9318	1.1055	1.1404	KStest_Ks_2sampleResult(statistic=0.37210713281221283, pvalue=2.66453525910375e-15)
Gene:Nkx2-5	1.6377	1.1091	1.116	KStest_Ks_2sampleResult(statistic=0.236648498451324, pvalue=0.00010407105192573885)
Gene:Srf	0.8881	0.5547	0.5786	KStest_Ks_2sampleResult(statistic=0.3213593693219516, pvalue=3.61313609379e-08)
process/heart_17.5e_if_gene_promoter_matrix.txt				
Gene:Gat4	1.5781	0.8268	0.8461	KStest_Ks_2sampleResult(statistic=0.4407584982373154, pvalue=2.72004410313163e-14)
Gene:Me2a	1.6498	0.8774	0.9111	KStest_Ks_2sampleResult(statistic=0.377039104797474, pvalue=4.21884749170599e-15)
Gene:Nkx2-5	1.3959	0.8651	0.8763	KStest_Ks_2sampleResult(statistic=0.203480142969696, pvalue=9.7634516033734e-08)
Gene:Srf	0.7528	0.435	0.4684	KStest_Ks_2sampleResult(statistic=0.34308130608263867, pvalue=4.62272983011206e-09)
process/heart_18.5e_if_gene_promoter				