The 3D genome and predictive gene regulatory models

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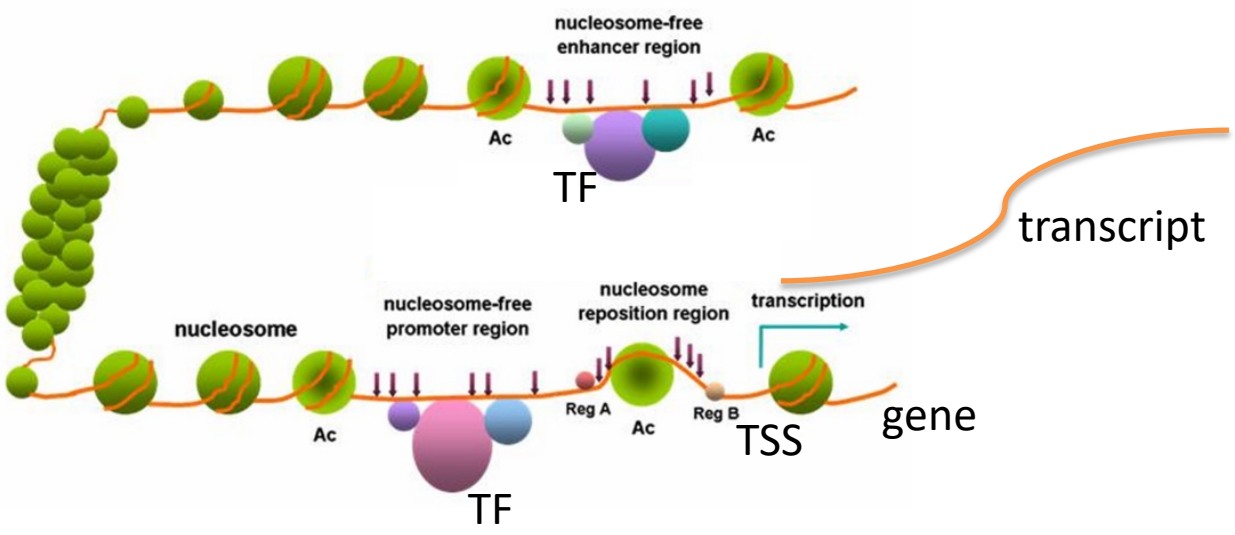
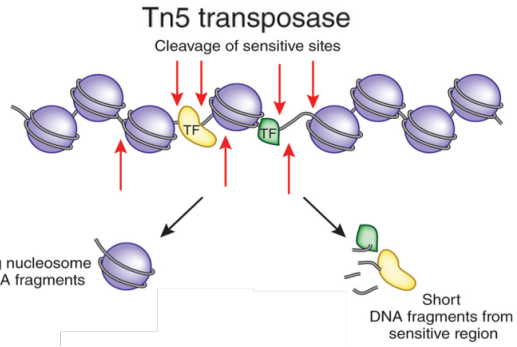
Deciphering gene regulation

- Transcriptional regulation coordinated by transcription factors (TFs) binding to promoter and enhancer elements
- Distal enhancers may be >1Mb from promoters, physically interact via chromatin looping
- 1D epigenomic data (chromatin accessibility, histone marks) map presence of candidate enhancer elements but not their connectivity



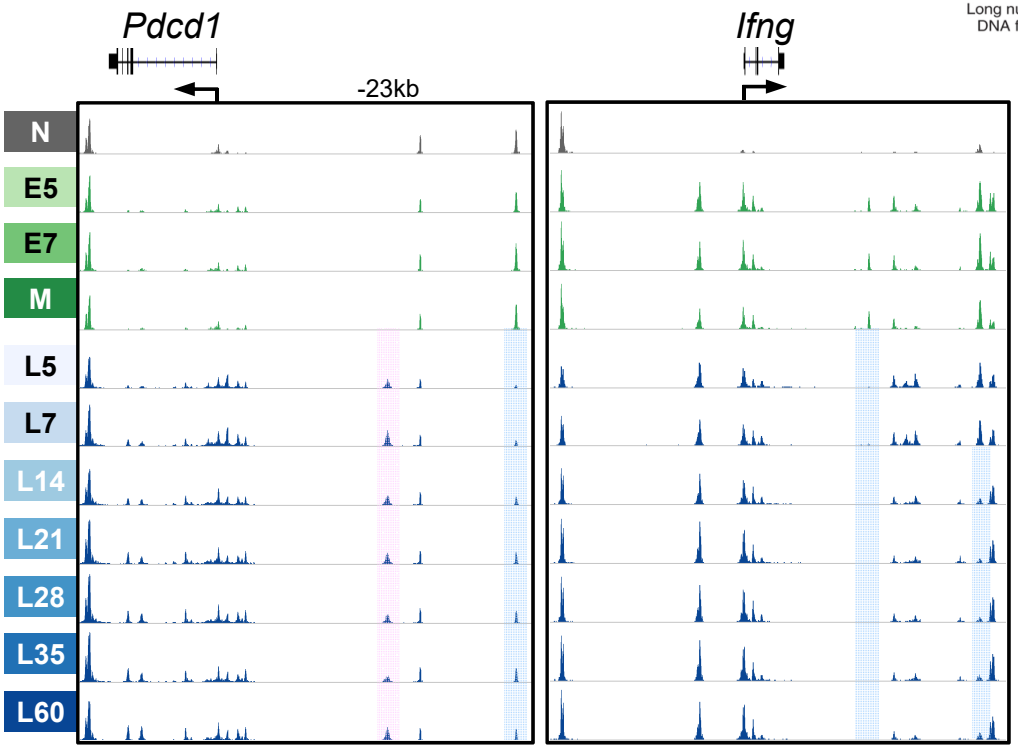
Epigenomic data encodes regulatory information

- E.g. chromatin accessibility (ATAC-seq) maps loca regulatory elements and encodes global differentiation state



Functional CD8 T cells

Tumor-specific dysfunctional CD8 T cells



low *Pdcd1*,
high *Ifng* expression

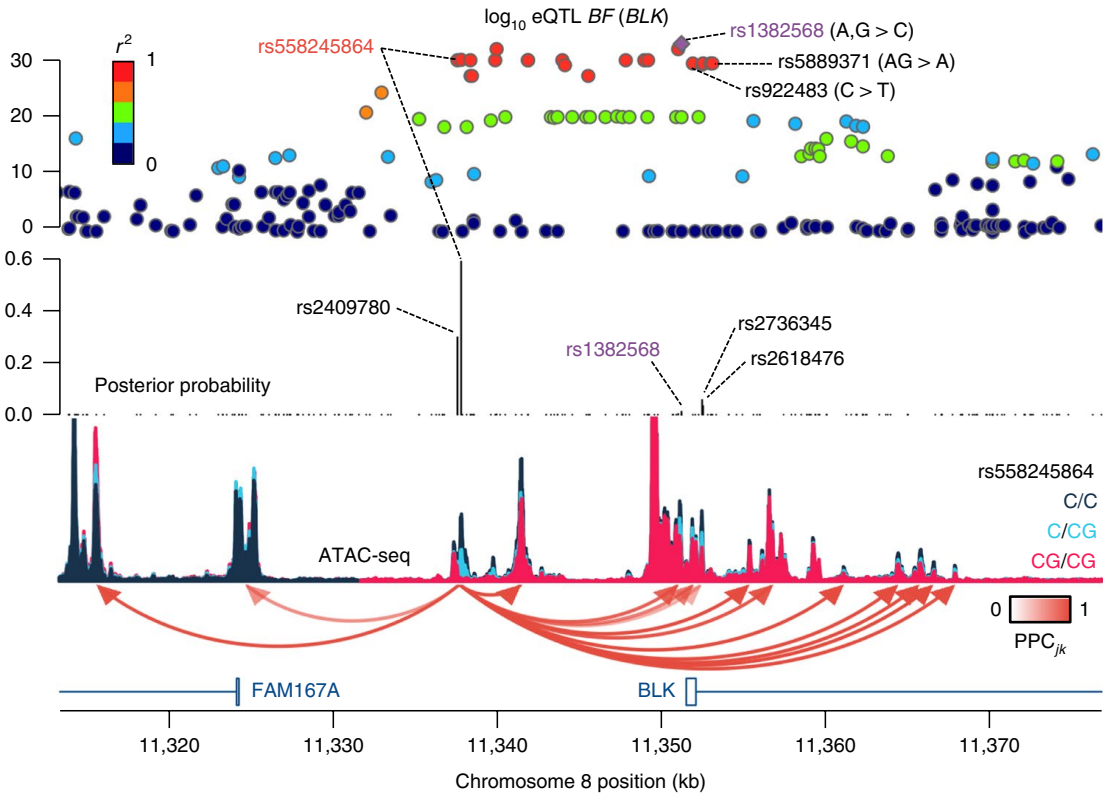
gain of *Pdcd1*,
loss of *Ifng* expression

Philip et al., Nature 2017

Ascribing function to non-coding genetic variants

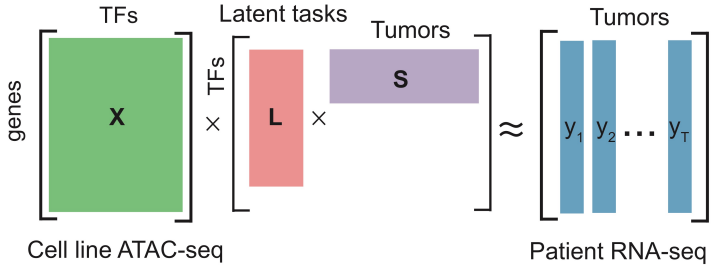
- Most GWAS signals reside in non-coding regions, causal variant assumed to be regulatory, i.e. alter regulation of target gene (possibly quite distal)
- Predictive models of gene regulation could infer the role of genomic elements, individual genetic variants on target gene expression

Example from Kumasaka et al., Nat Genet 2019

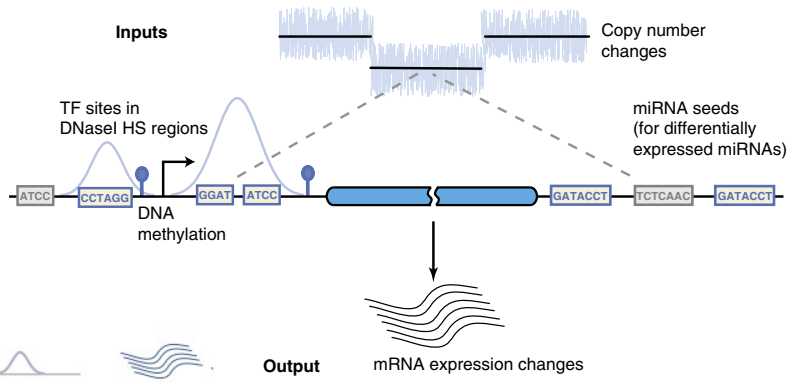


Predictive gene regulatory models

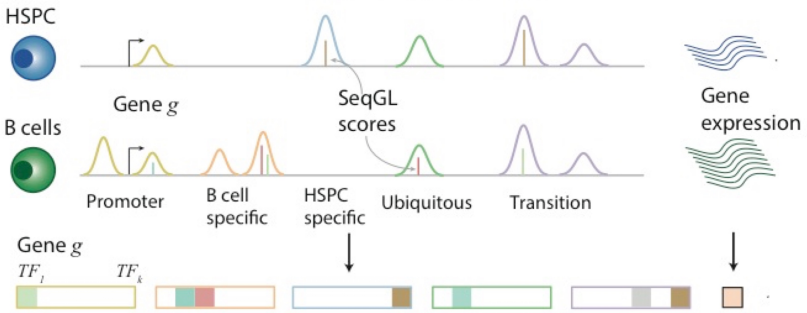
- Previous GRMs predict gene expression (or fold change) from DNA sequence and accessibility/activity of regulatory elements *in order to decipher gene regulation*



Osmanbeyoglu et al., Nat Commun 2019



Setty et al., Mol Syst Biol 2012

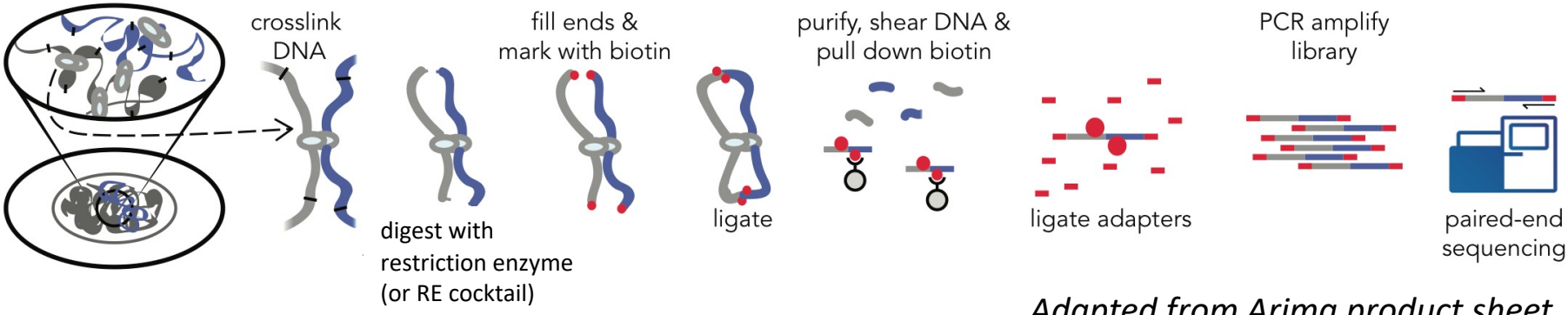


Gonzalez, Setty* et al., Nat Genet 2015*

- Missing information: *connectivity* of promoter and enhancers
- Idea: use 3D interaction data in graph neural network GRMs

Mapping the 3D genome

- Hi-C, chromosome conformation capture
  - Capture 3D interactions: crosslink DNA (now in situ), restriction enzyme digest, proximity ligation, pull down, paired-end sequencing

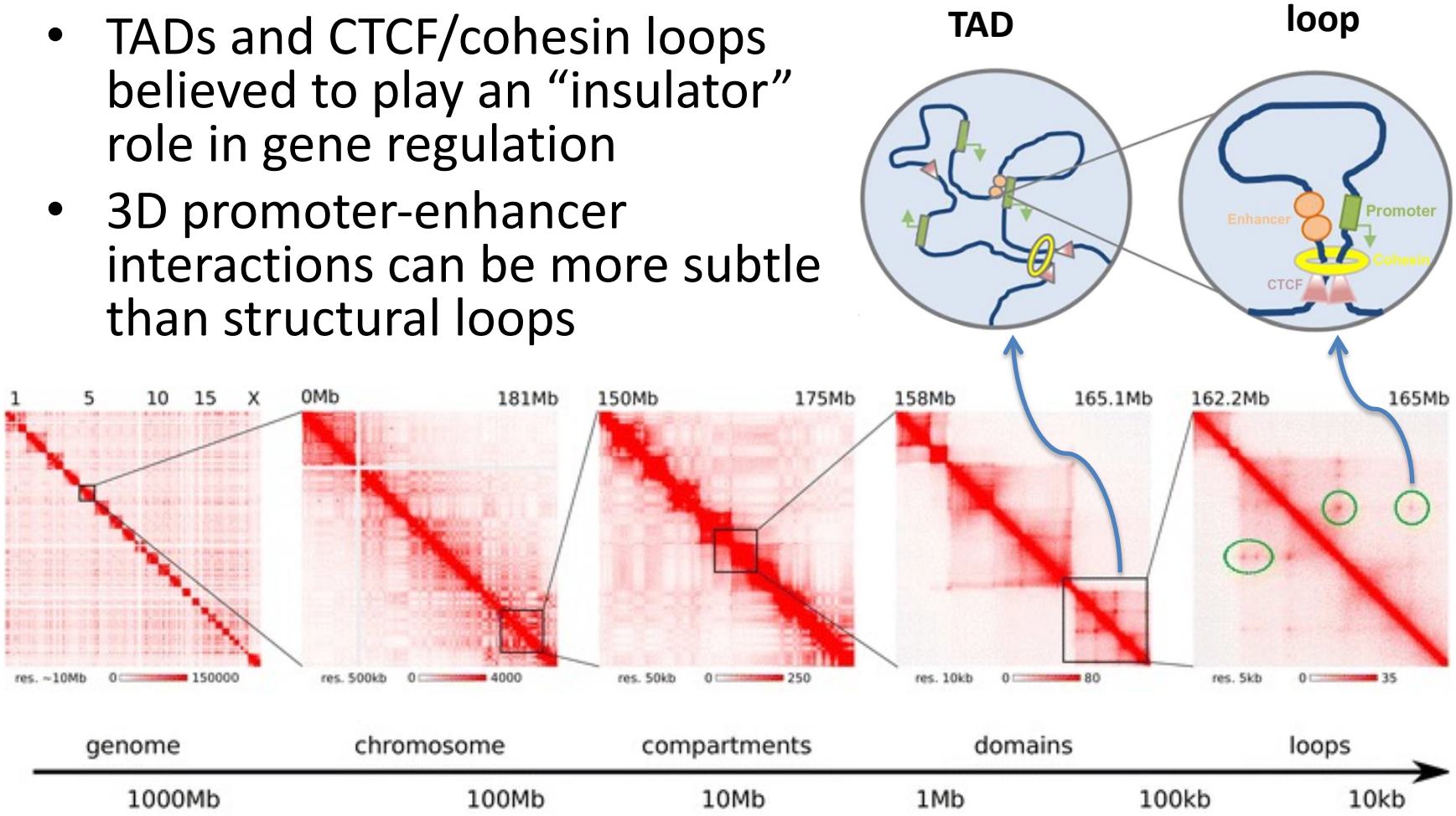


Adapted from Arima product sheet

  - Read pair = “contact”; build contact matrix for input cell population

Hierarchical folding of chromatin

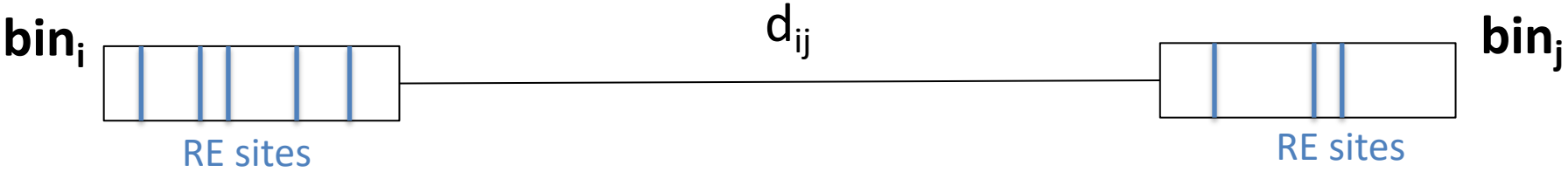
- TADs and CTCF/cohesin loops believed to play an “insulator” role in gene regulation
- 3D promoter-enhancer interactions can be more subtle than structural loops



Adapted from Wright et al., 2019

Methods matter: HiC-DC+

- "Hi-C direct caller": use read counts from raw contact matrix directly, without normalization
  - Estimate background model (expected read count) directly from data using negative binomial regression
  - Covariates: genomic distance (spline fit), mappability, effective bin size (related to restricting enzyme density), GC content
  - Assign *P* value (or *Z*-score) to interactions

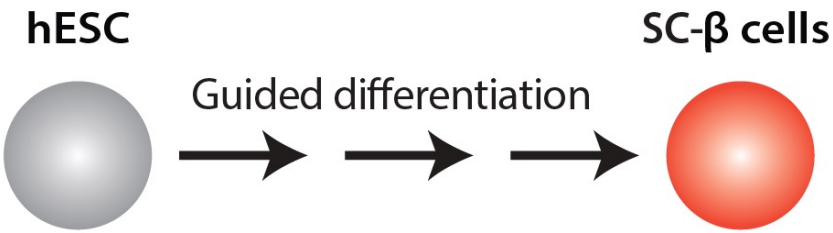


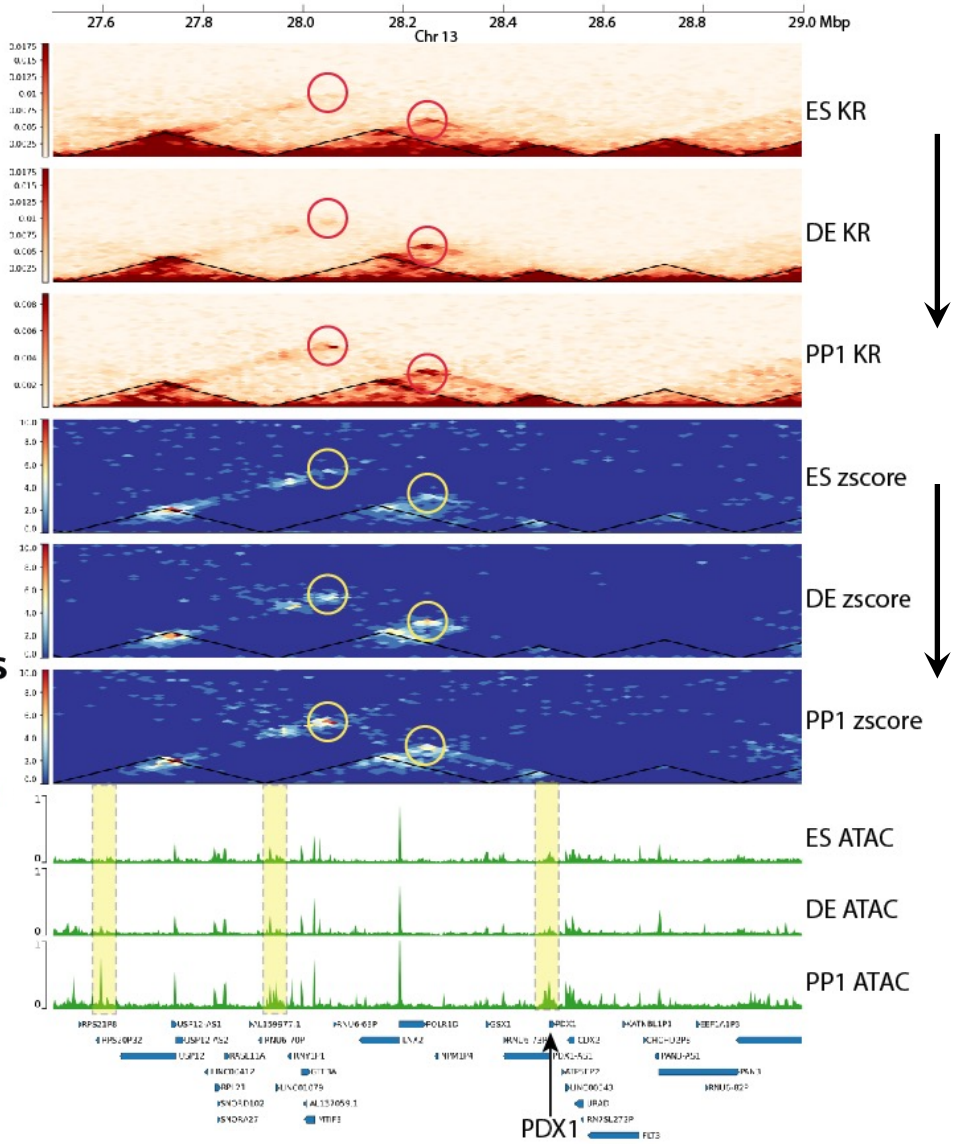
- HiC-DC+: Efficient code, extends to HiChIP, *differential* interactions between cell types

Carty et al., Nat Commun 2017;
Sahin et al., in revision, bioRxiv 2020

Methods matter: HiC-DC+

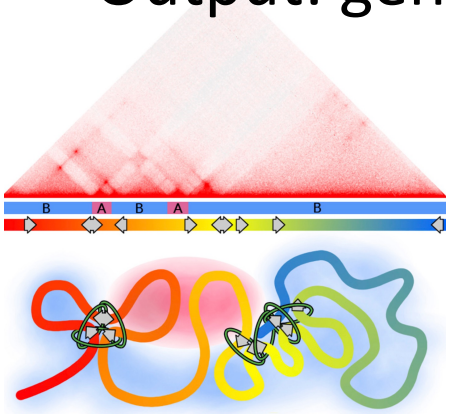
- Gain of promoter-enhancer for diabetes gene *PDX1* in guided pancreatic differentiation
  - With Danwei Huangfu and Eftychia Apostolou (as 4D Nucleome project)

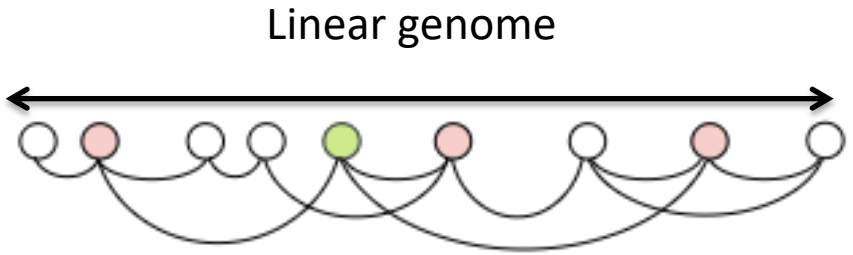




GraphReg: graph neural networks for gene regulatory models

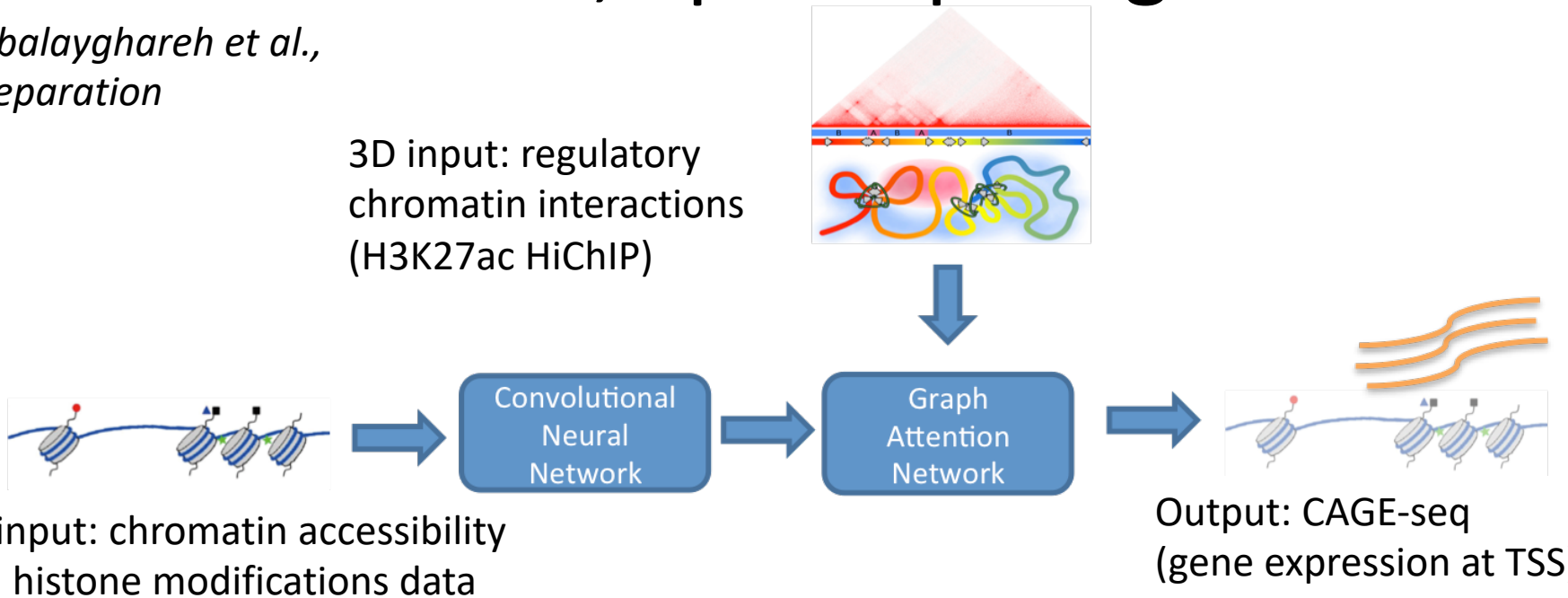
- Idea: use Hi-C/HiChIP to encode long-range chromatin interactions as a graph, propagate information information via graph neural networks (GNNs)
- Nodes of graph = genomic bins, edges = 3D genomic interactions
- Input features: epigenomic data or DNA sequence
- Output: gene expression (at node)





Epigenome-based gene regulatory model, Epi-GraphReg

Kharbalayghareh et al., in preparation



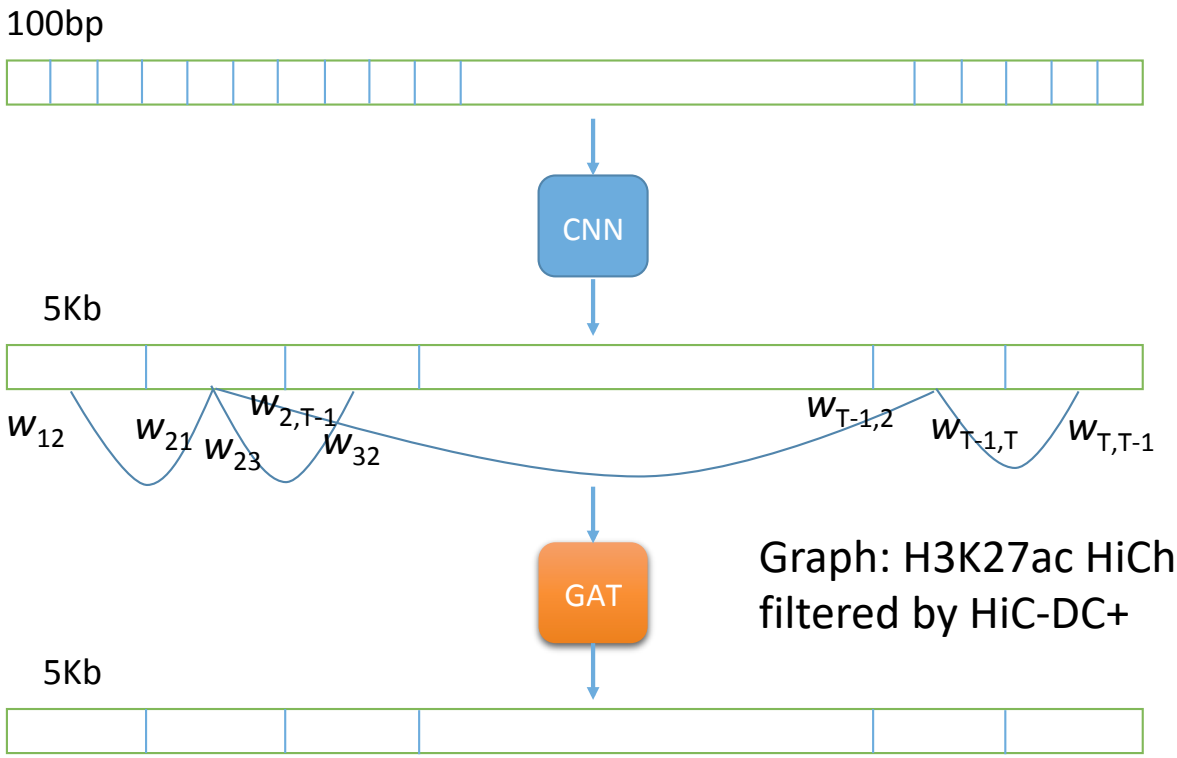
- Predict gene expression from *activity* and *connectivity* of regulatory elements
- "Cell type agnostic": can generalize to a new cell type given cell-type specific 1D and 3D inputs

Epi-GraphReg architecture

Inputs: DNase-seq, H3K4me3 (promoter mark), H3K27ac (enhancer mark)

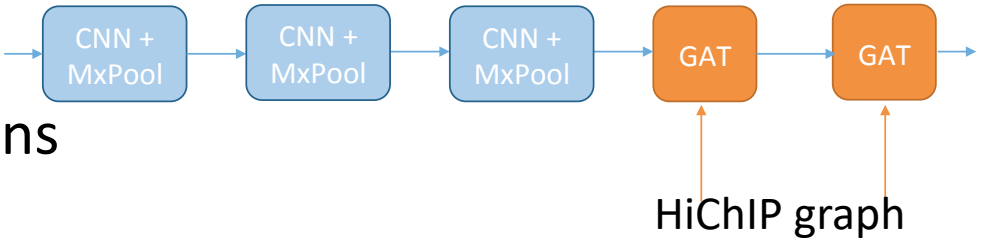
GAT learns to weight edges

Output: CAGE-seq



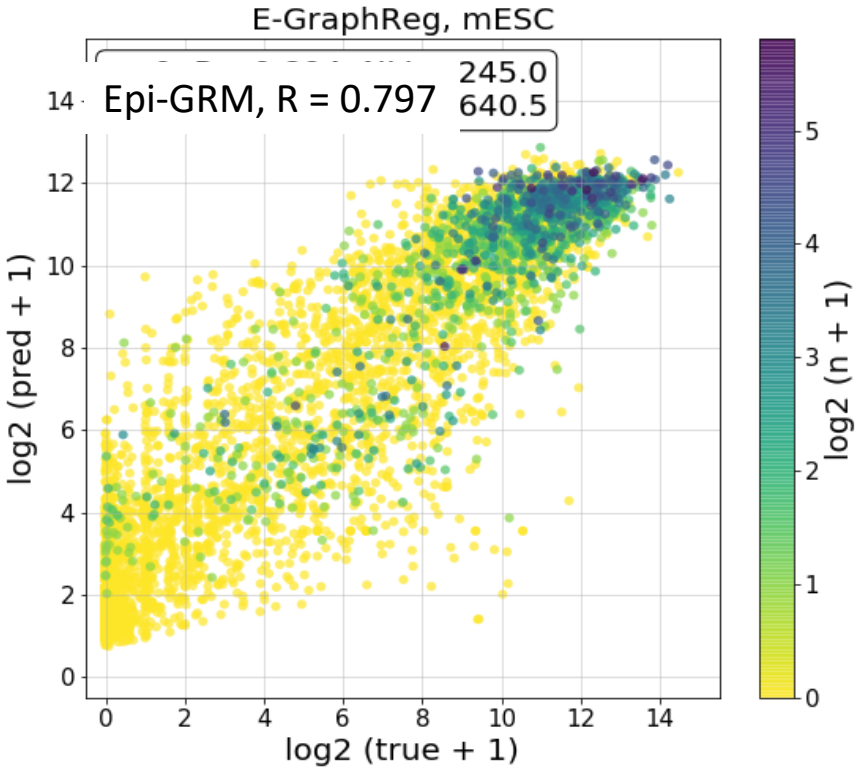
Graph: H3K27ac HiChIP, filtered by HiC-DC+

- Train on 6Mb input regions
- Poisson loss on middle 2Mb bins

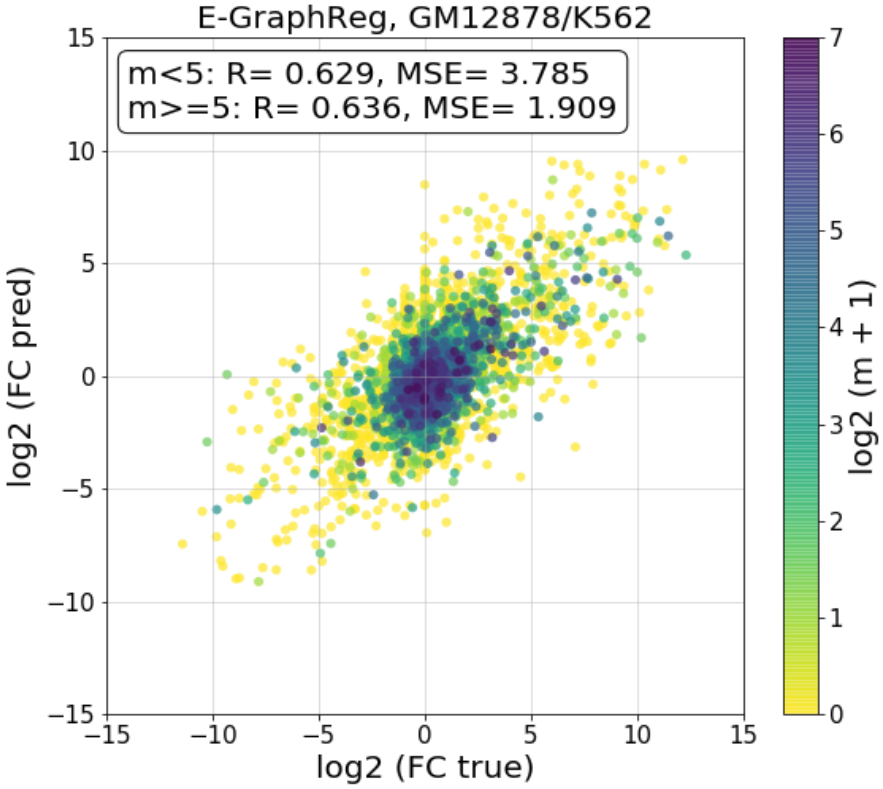


Prediction of gene expression

- Train on cell line data, assess performance on held-out chromosomes



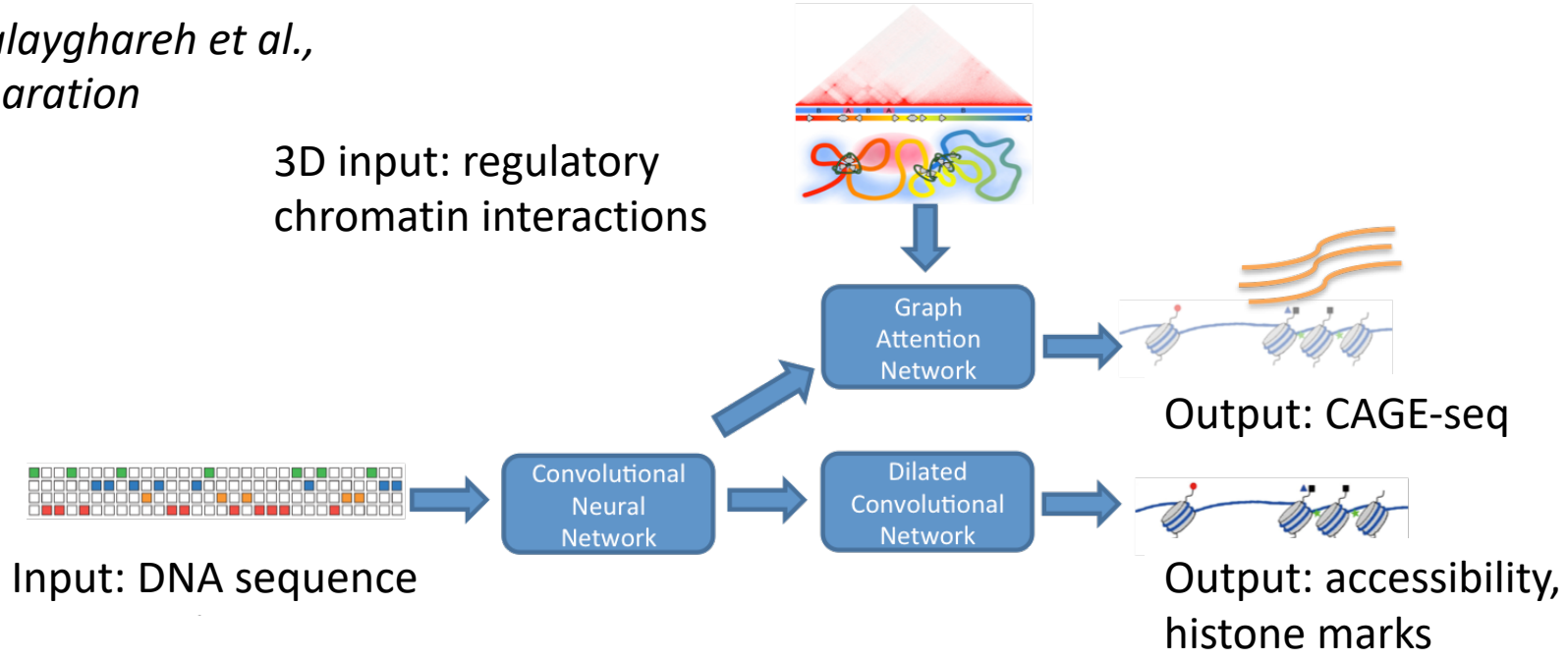
mESC expression



GM12878 vs. K562
log fold change

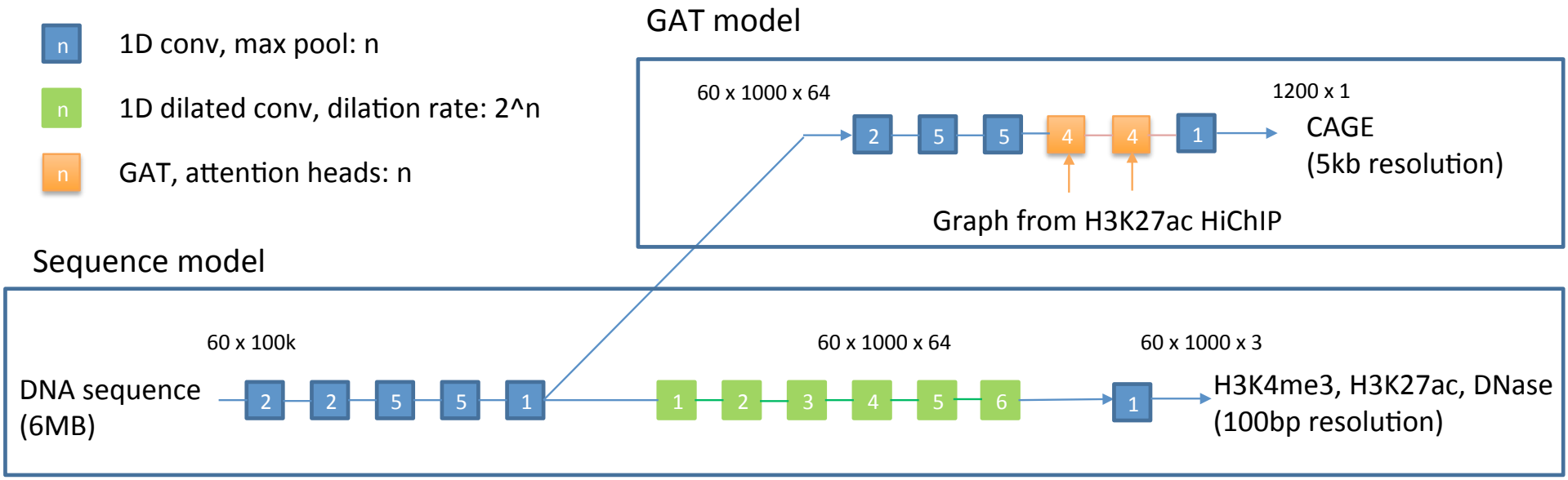
Sequence-based gene regulatory model, Seq-GraphReg

Kharbalayghareh et al., in preparation



- Predict expression and 1D epigenomic signals from genomic DNA sequence + 3D connectivity
- “Cell type specific”: captures TF binding signals that are specific to the training cell type

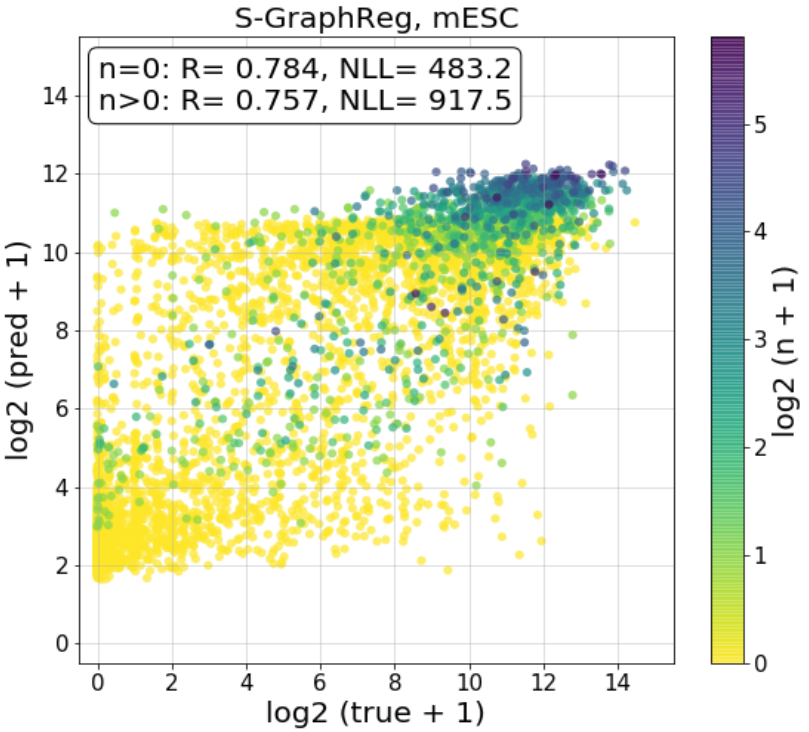
Seq-GraphReg architecture



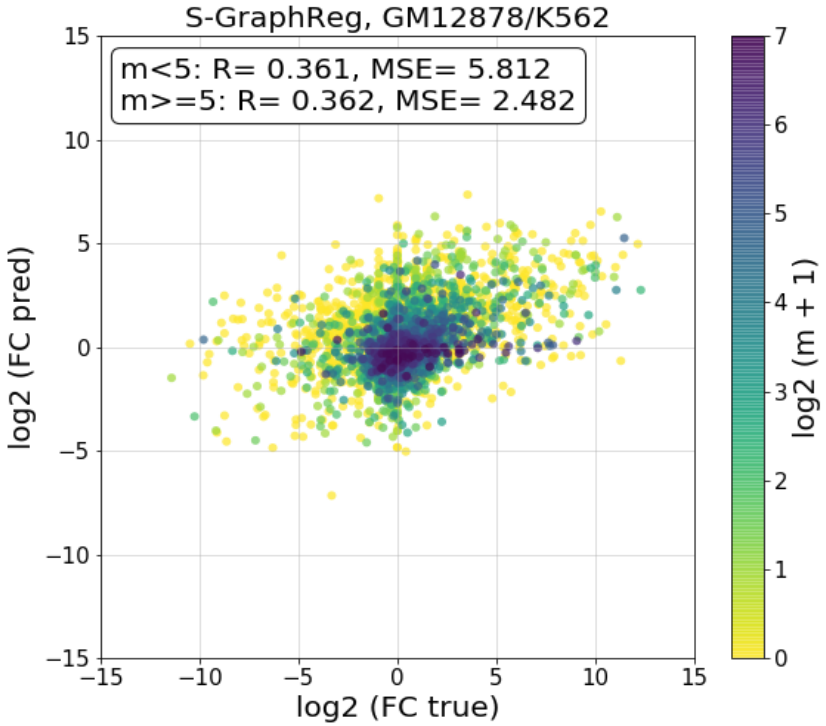
- Sequence-to-1D-epigenome component of the model is similar to Basenji (Kelley et al., 2018)
- Learn DNA sequence features that predict regulatory element activity, combined over HiChIP graph to predict expression

Prediction of gene expression

- Train on ENCODE GM12878 and K562 cell line data, assess performance on held-out chromosomes

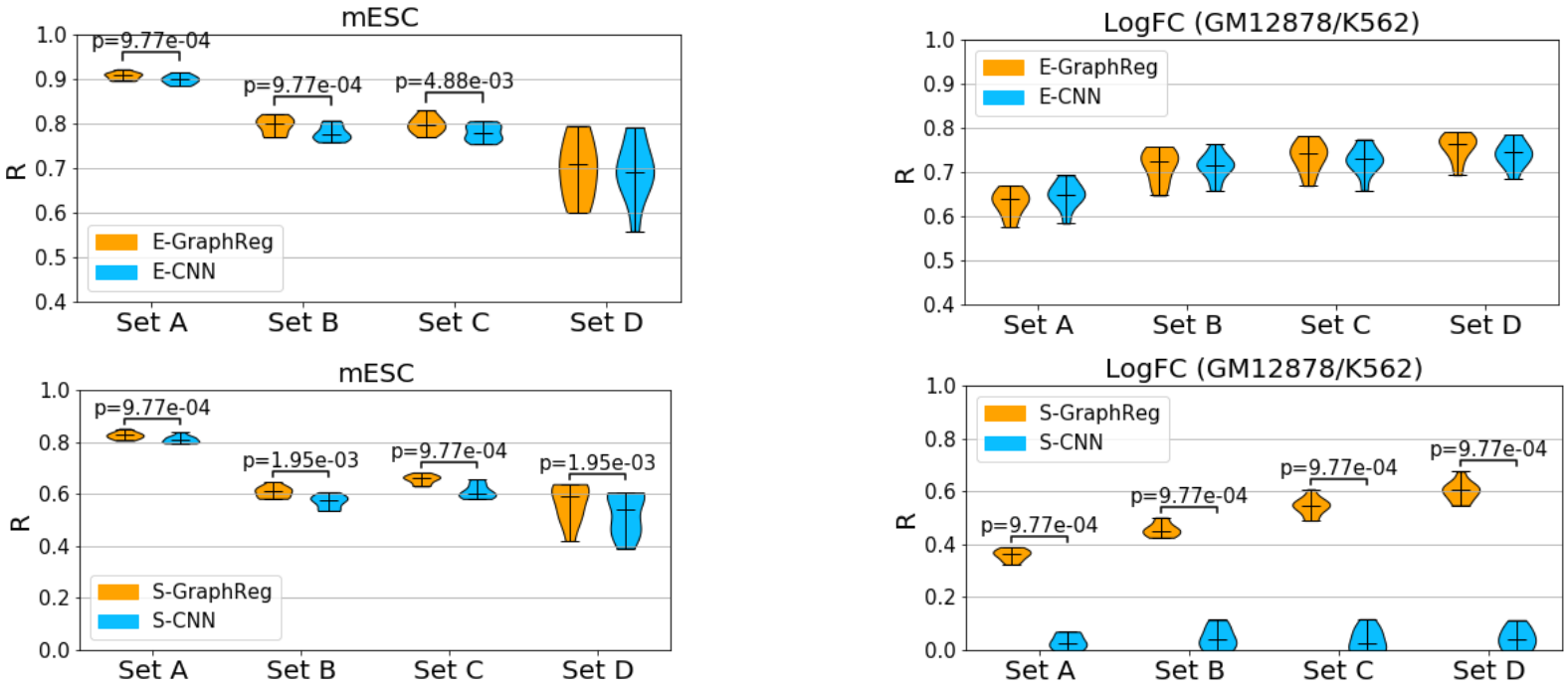


mESC expression



GM12878 vs. K562
log fold change

Prediction performance

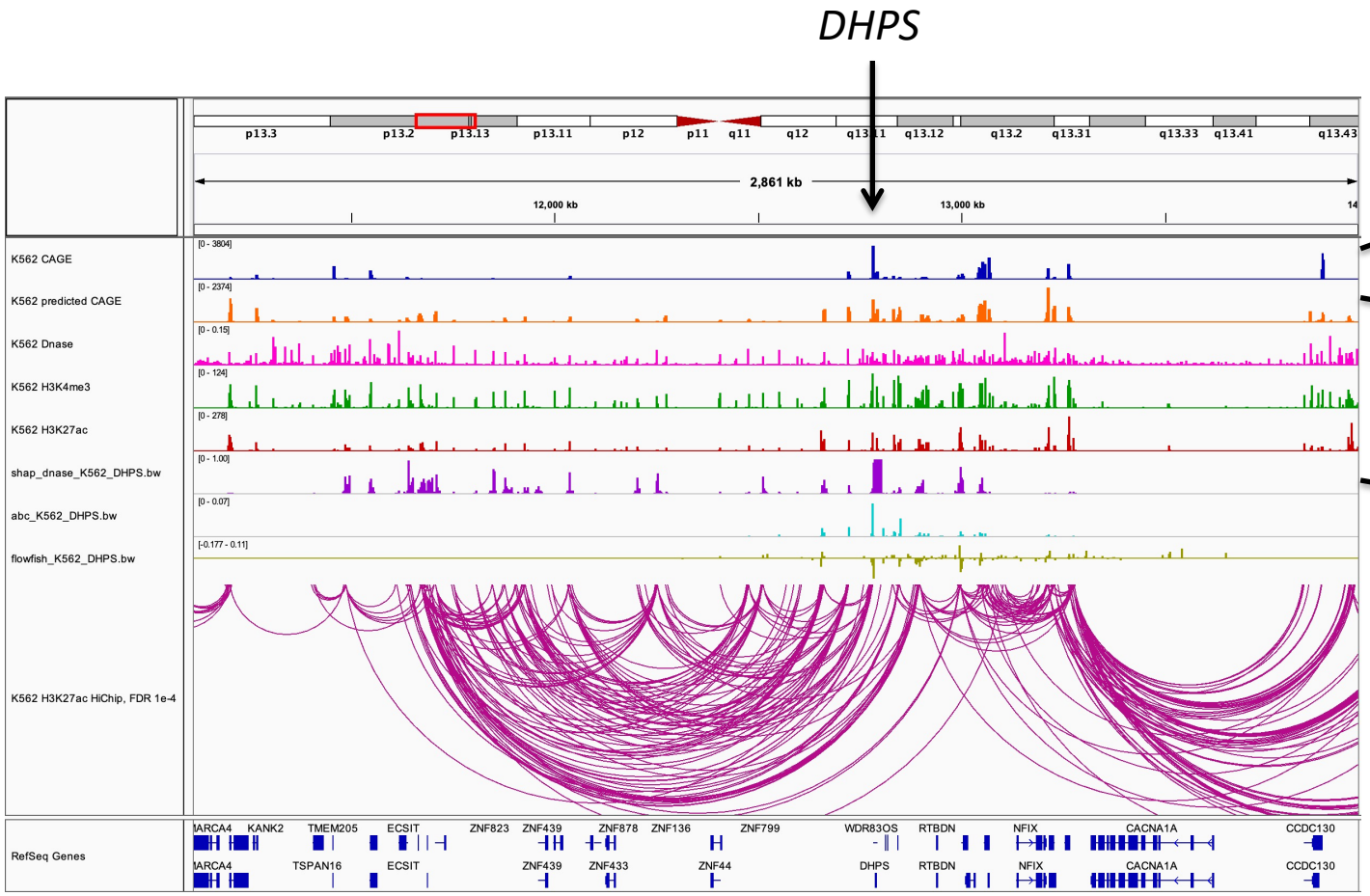


A: all genes, B: all expressed genes, C: expressed genes at least 1 HiChIP edge, D: expressed genes with at least 5 HiChIP edges

- Graph NN models outperform baseline sequence models (1D dilated CNNs) in all cases
- Sequence-based prediction is more difficult
- Prediction of expression *per se* is not the point: want to interpret the model

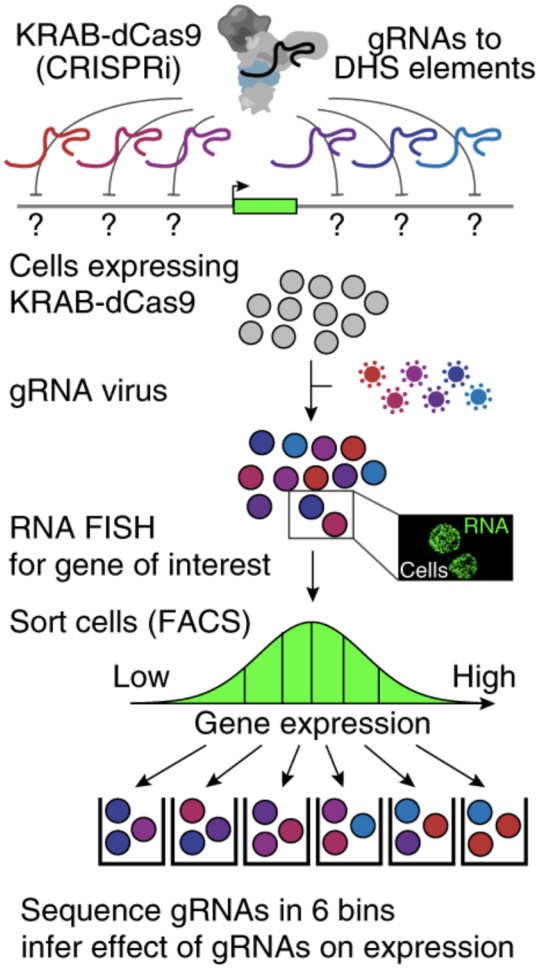
Feature attribution to predict functional enhancers

- DeepSHAP identifies features/genomic bins that contribute most to specific gene predictions



Epi-GraphReg

Evaluation of enhancer prediction with FlowFISH

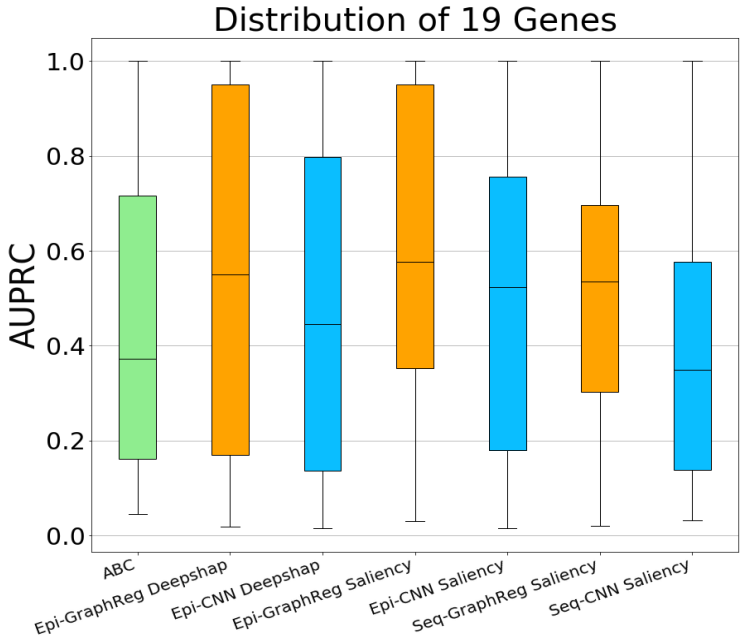


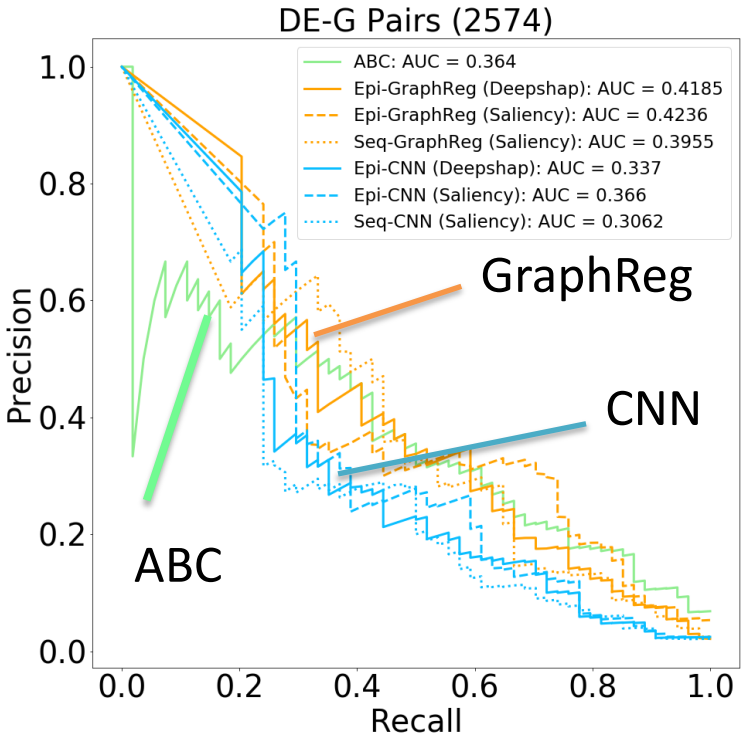
Fulco et al., Nat Genet 2019

- CRISPRi-FlowFISH: CRISPR inactivation screen against candidate enhancers, reads out expression change of target gene
- Activity-by-contact (ABC): score for predicting functional enhancers based on activity (DNase, H3K27ac) and Hi-C contacts

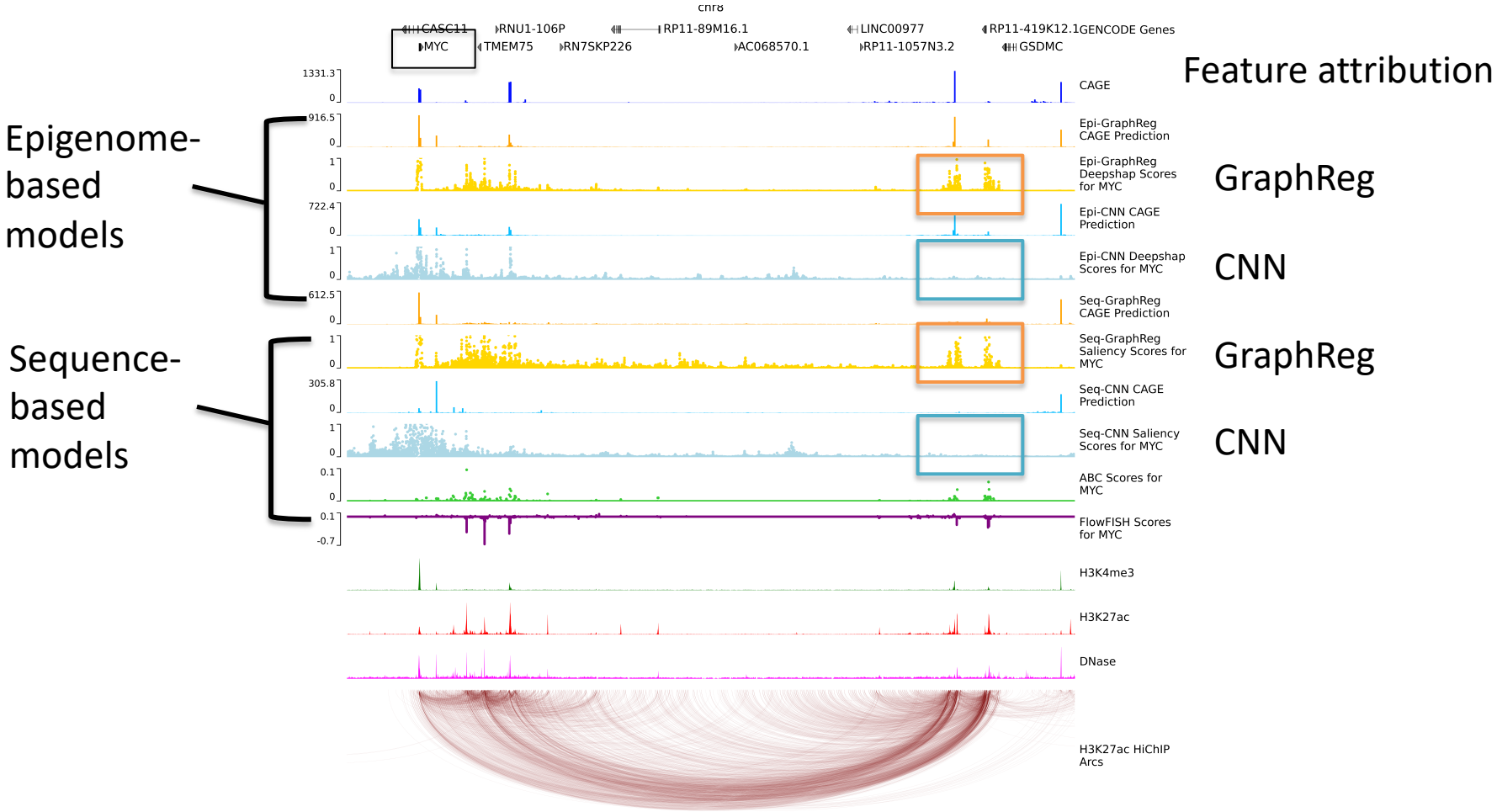
GraphReg improves functional enhancer prediction

- Use FlowFISH experiments sufficient data on distal elements (2906 candidate elements for 21 genes)
- GraphReg models with DeepSHAP or saliency outperform CNN models, ABC





GraphReg models access distal information unavailable to CNNs



- Dilated CNN has wide receptive field, but feature attribution shows they rely on promoter-proximal inputs

Conclusions

- Graph neural network model can predict gene expression (TSS output) across large genomic regions from 3D and 1D data, or from DNA sequence using 1D epigenomic prediction as auxiliary task
- Epi-GraphReg and Seq-GraphReg outperform baseline dilated 1D CNN models for gene expression prediction
- More importantly, can use feature attribution to predict functional enhancers for genes
- GraphReg outperforms CNN models and ABC score for identifying enhancer elements, as validated by CRISPRi-FlowFISH

Rapid developments in machine learning, epigenomics/3D genomics, and genome editing enable advances in modeling and deciphering gene regulation

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