Understanding transcriptional regulation by integrative analysis of transcription factor binding data

Cheng et al. 2012

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Introduction

DNA-binding Proteins

- sequence-specific TFs (TFSS): MYC, MAX
- general or nonspecific TFs (TFNS): TBP (TATA-binding proteins)
- chromatin structure factors (ChromStr): CHD2
- chromatin remodeling factors (ChromRem)
- histone methyltransferases (HISase)
- Pol3-associated factors (Pol3F): POLR3A

Gene expression

- is the process of producing a specific amount of gene product in a spatiotemporal manner.
- is regulated in steps including: transcriptional regulation, splicing, end modification, export, and degradation.
- Transcriptional regulation can occur on both genetic and epigenetic levels.

Questions

- Is there much difference in the prediction accuracy of expression levels of TSSs captured by different technologies(CAGE,RNA-PET,RNA-seq)
- What is the effect of promoter CpG content on gene expression?
- Do TFs regulate alternative TSSs in the same mechanisms?
- Between two cell lines, can the difference of TF-binding signals precisely reflect the differential expression of TSSs?
- Can TF-binding signals predict histone modifications?

Data and Models

ENCODE data

- Gene expression data (TSS):
  - >130,000 TSSs; 267 expression profiles; 12 cell lines (K562 and GM12878)
  - CAGE, RNA-seq, RNA-PET
- TF binding data:
  - >120 TFs; >400 binding profiles
  - ChIP-seq

Machine Learning Models

- Four methods:
  - multiple linear regression (MLR)
  - multivariate adaptive regression splines (MARS)
  - support vector regression (SVR): single predictor
  - random forest (RF): multiple predictors
- Evaluation:
  - Regression: R; R^2
  - Classification: AUC
  - 2000 promoters training; rest testing

Nonlinear relationship between TF binding and TSS expression

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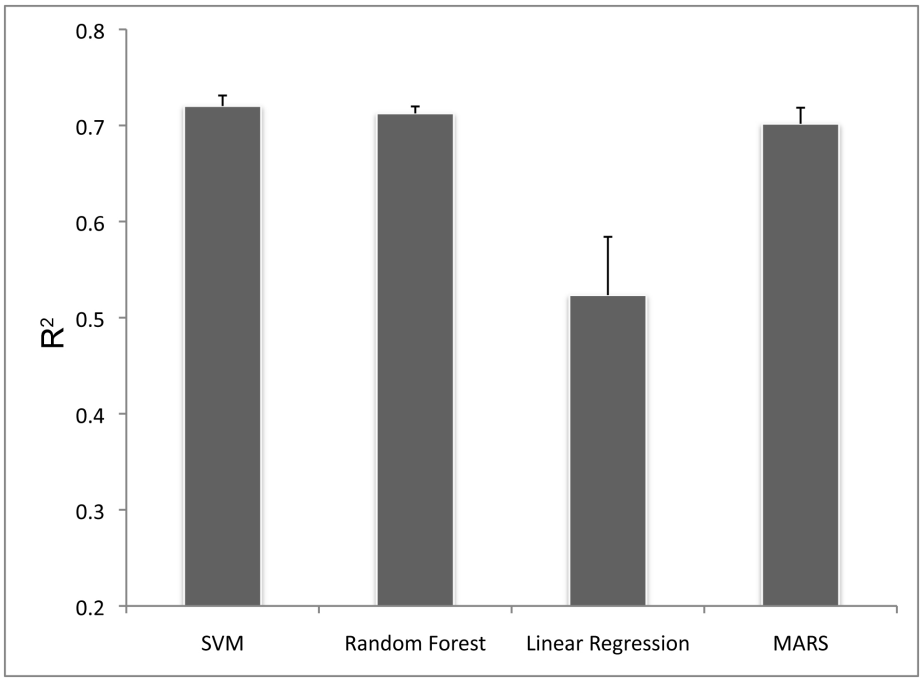
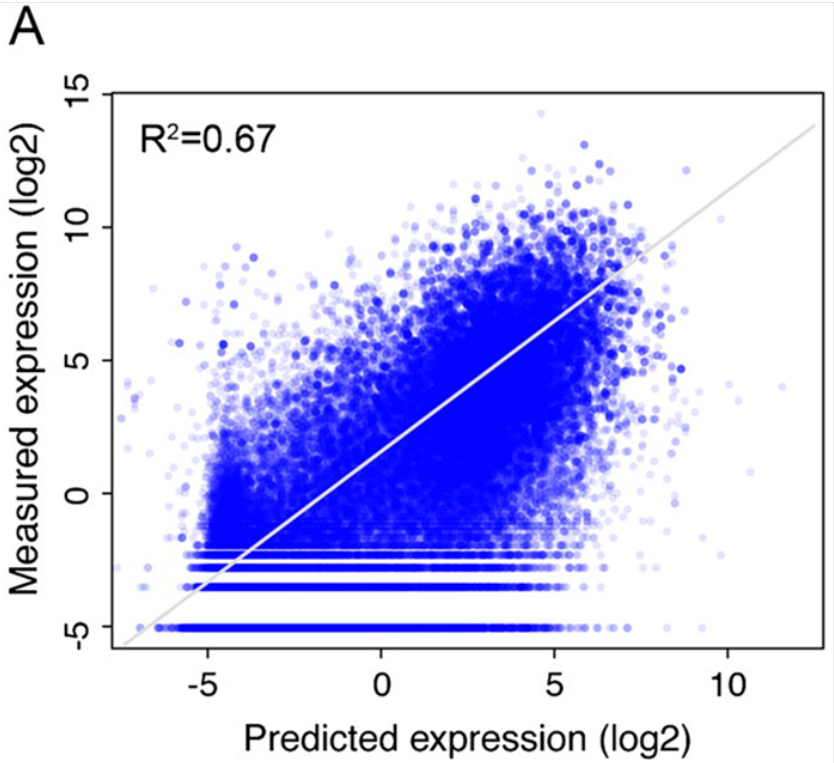


- SVR: single predictor
- RF: multiple predictors
- CAGE ployA+ whole cell from K562 (Default)

Results

Accuracy of the TF model for predicting TSS expression levels



- Figure 1.A shows the consistency between predicted and actual expression levels of TSSs measured by CAGE of whole cell Poly A+ RNA in K562 cells. "Prediction accuracy" model.

Comparison of three different technologies

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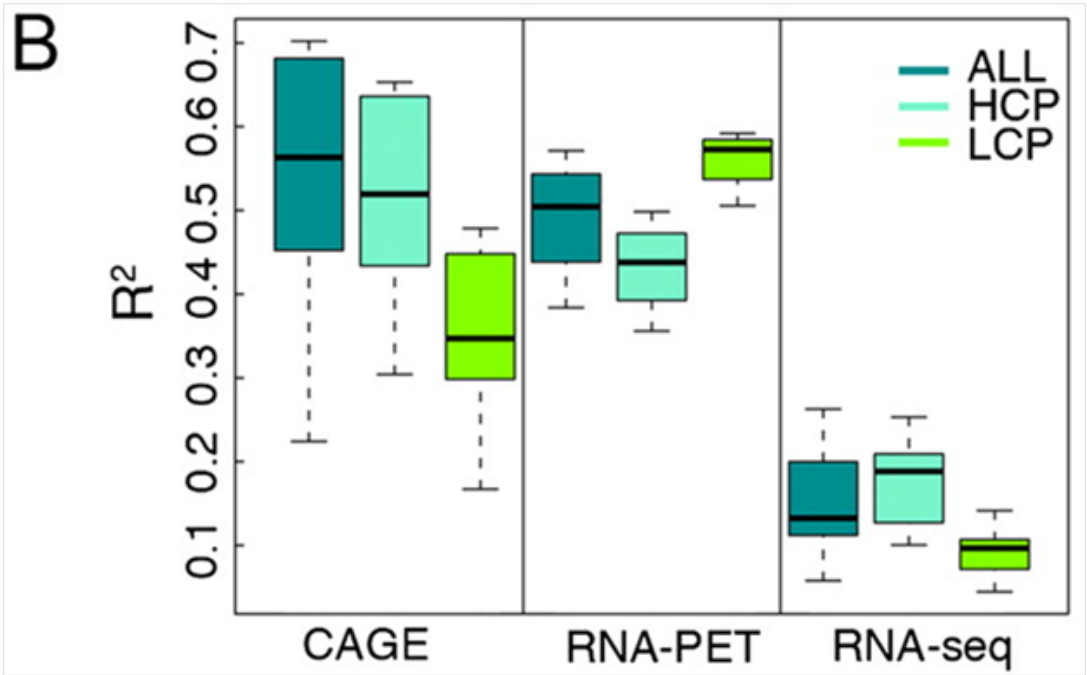

- They used the binding signals of 40 TFs to predict each of the 57 K562 expression profiles
- The highest predictive accuracy was achieved from CAGE

Comparison of different RNA extraction protocols, different cellular components

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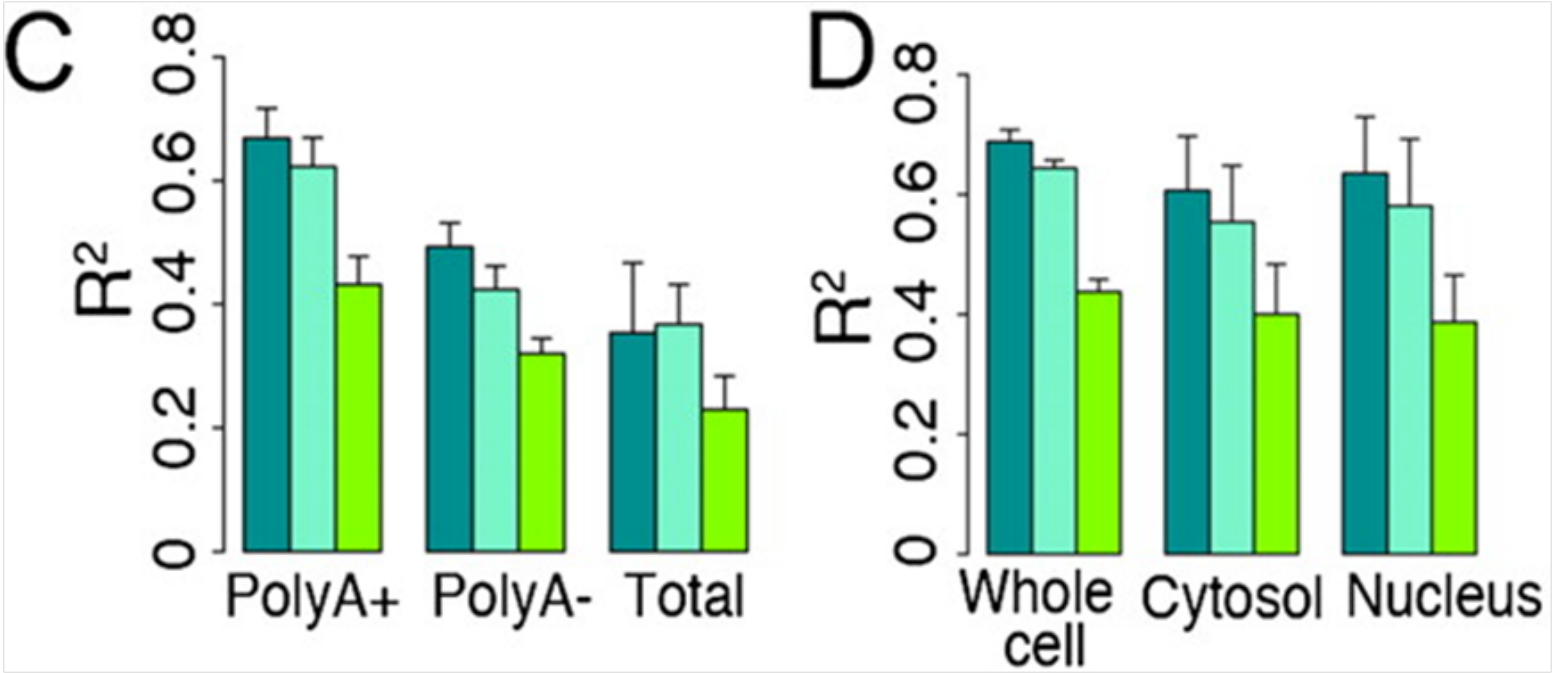

- protocols: Poly A+ >Poly A- >Total RNA
- cellular components: no obvious difference

The capabilities of different TFs to predict TSS expression level

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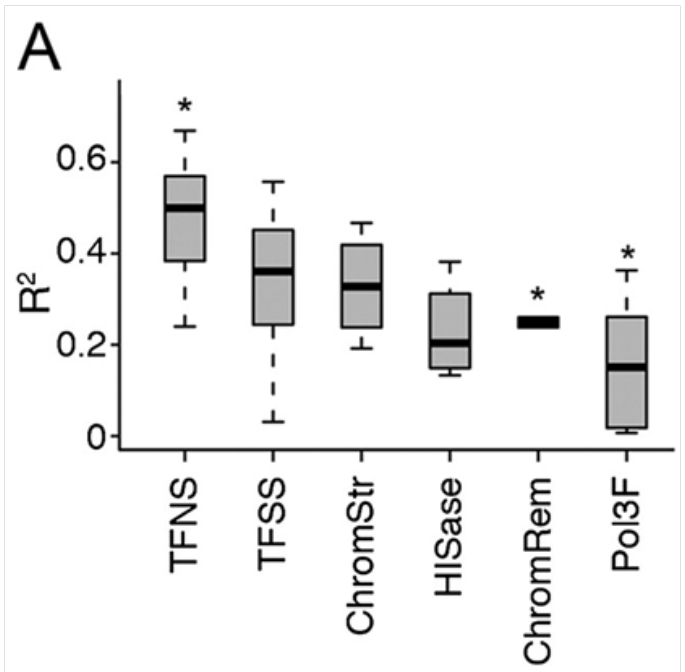
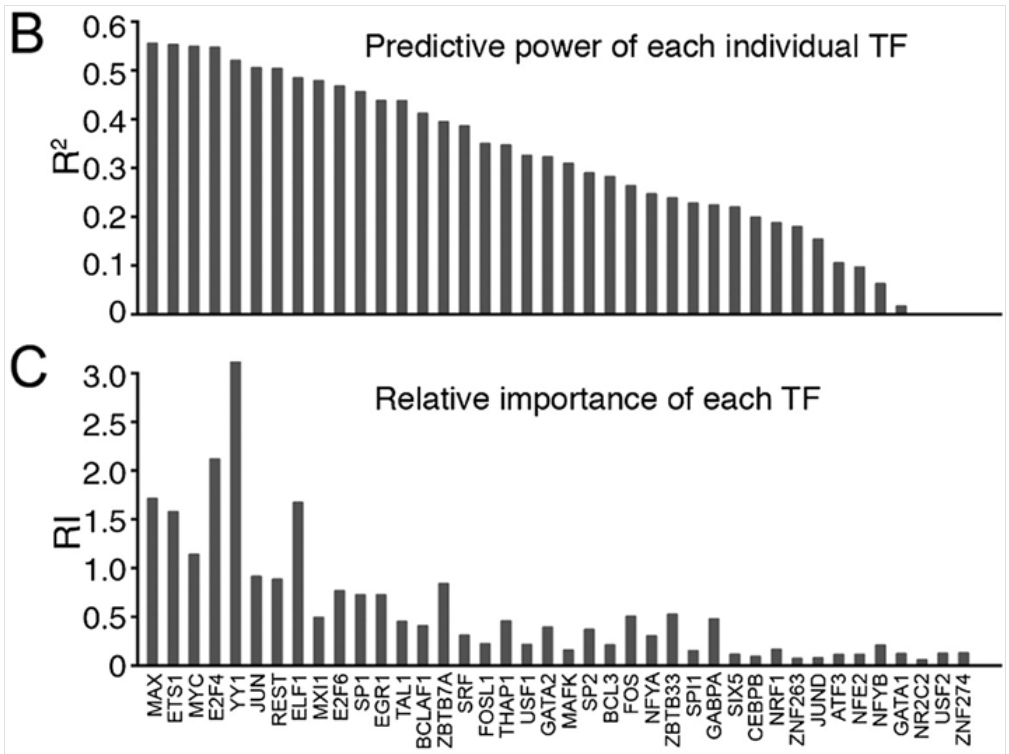


- TFNS TFs are the most predictive. (Binding of these TFs is essential for transcriptional initiation of most promoters)
- Pol3F are the least predictive. (RNA Pol III is involved in a small fraction of promoters)

The capabilities of individual TFSS TF to predict TSS expression level



- R^2 for each TF is fairly high.
- RI (increase of MSE when testing data permuted)

The relationship between promoter CpG content and expression level

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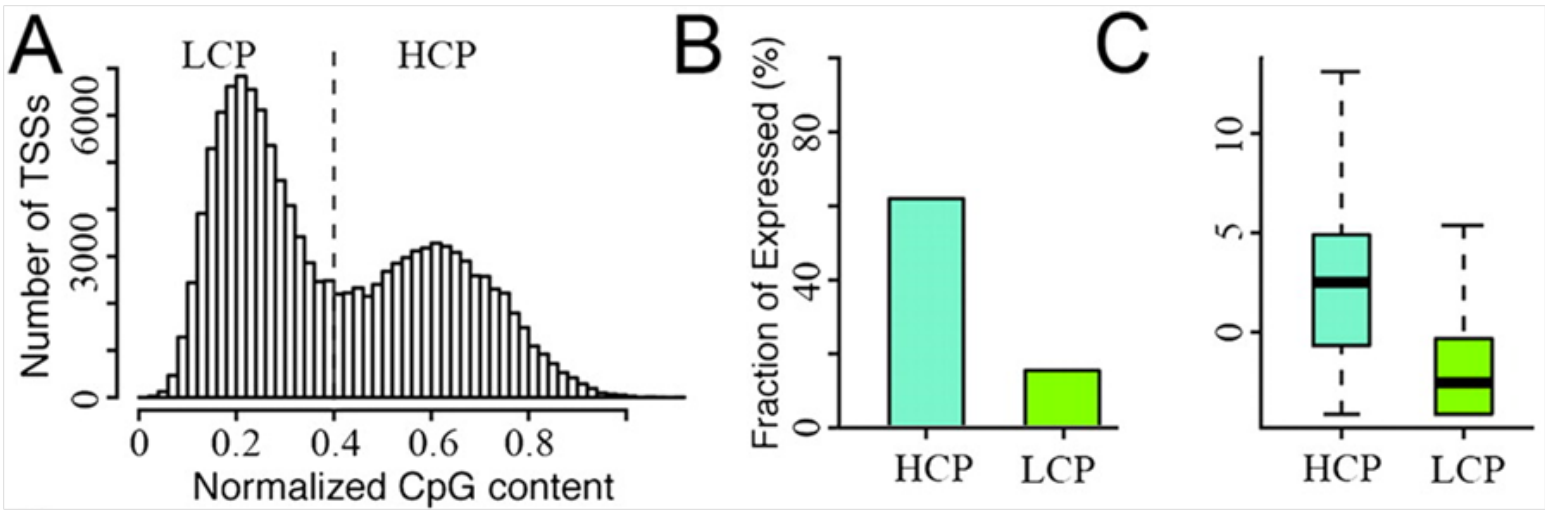

- A: Bimodal distribution: LCP and HCP
- B: HCP are more highly expressed than LCP.
- C: Among expressed TSSs, expression level HCP >LCP

Relative Importance for each TF, HCP vs. LCP

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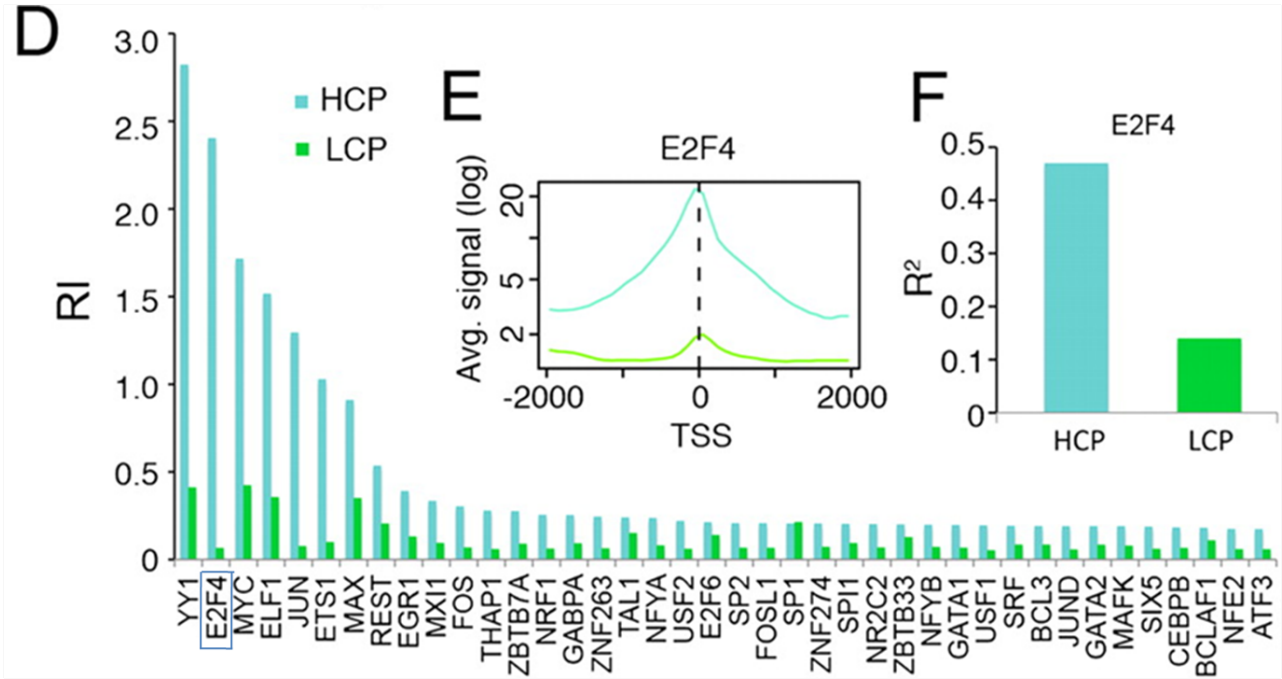

- D: RI: HCP >LCP; E2F4: high RI for HCP but low for LCP
- E: Binding signal of E2F4: HCP >LCP
- F: R^2 of E2F4 (single predictor): HCP >LCP
- The regulation of E2F4 on gene expression might be affected by status of CpG sites.

Correlation between CpG and expression level in different cell lines

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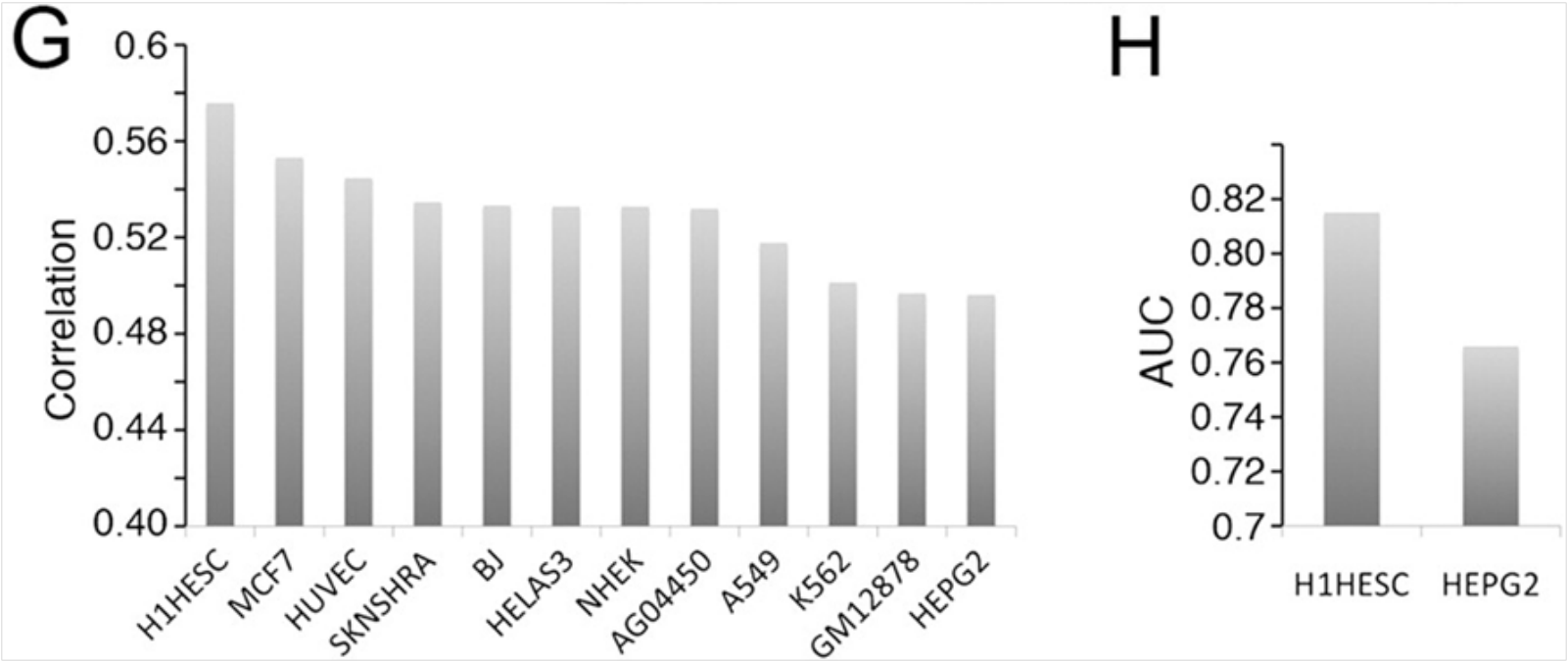

- G: Best correlation: H1HESC (H1 human embryonic stem cell)
- High CpG to UpG rate for promoters repressed in germline cells or in early developmental stage. CpG ->methylation ->expression repressed ->mutation ->lower CpG content?
- H: CpG as classifier for expressed or nonexpressed promoters. High accuracy: AUC=0.82 in H1HESC

Regulation of alternative TSS by TFs

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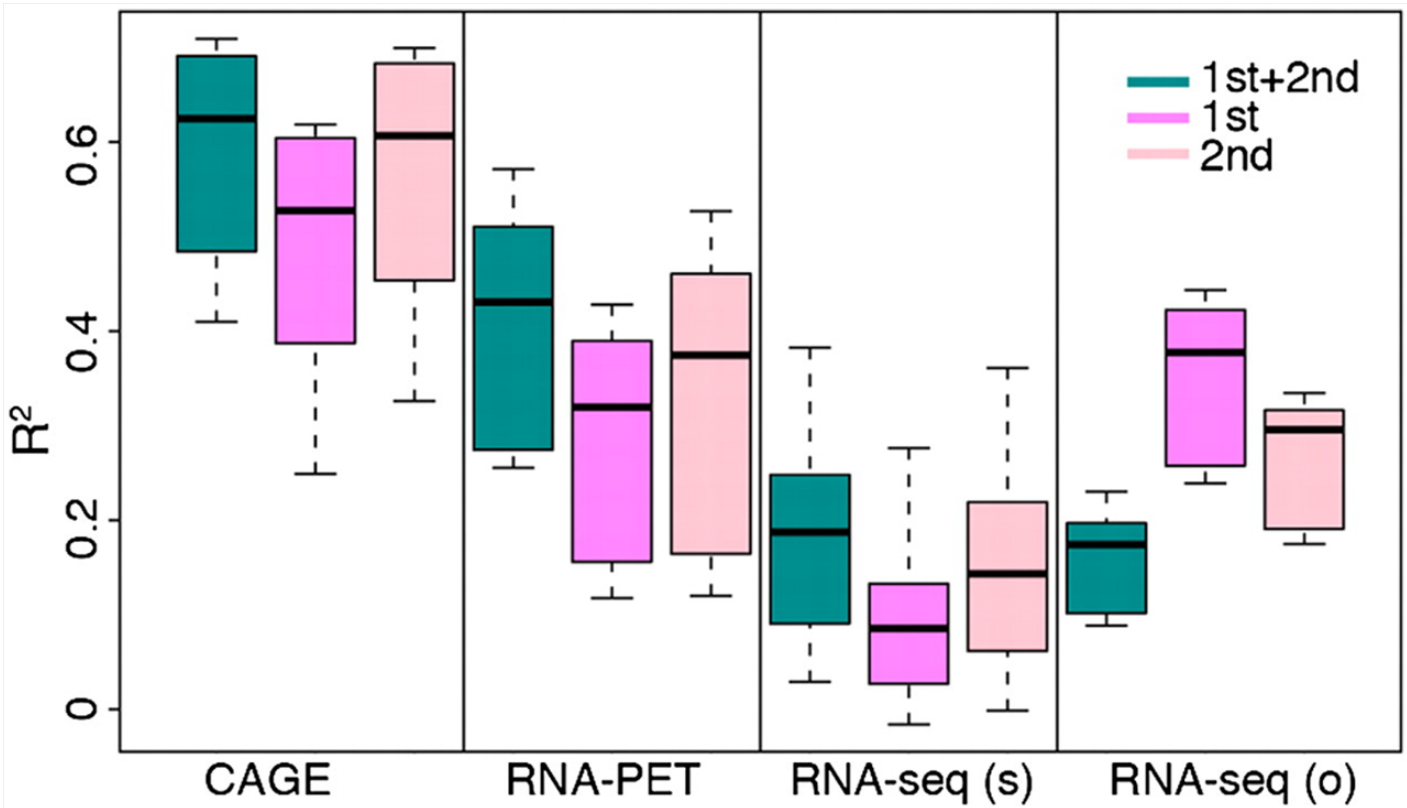

- Around 35% of GENCODE genes posses >1 TSS; compare 1st and 2nd TSS
- Higher predictive accuracy for 2nd TSS: CAGE, RNA-PET and RNA-seq(o)
- Expression levels of 1st and 2nd are similar ->2nd TSS rely more on TF regulation. Also different RIs.

Cell line specificity of the TF model 1

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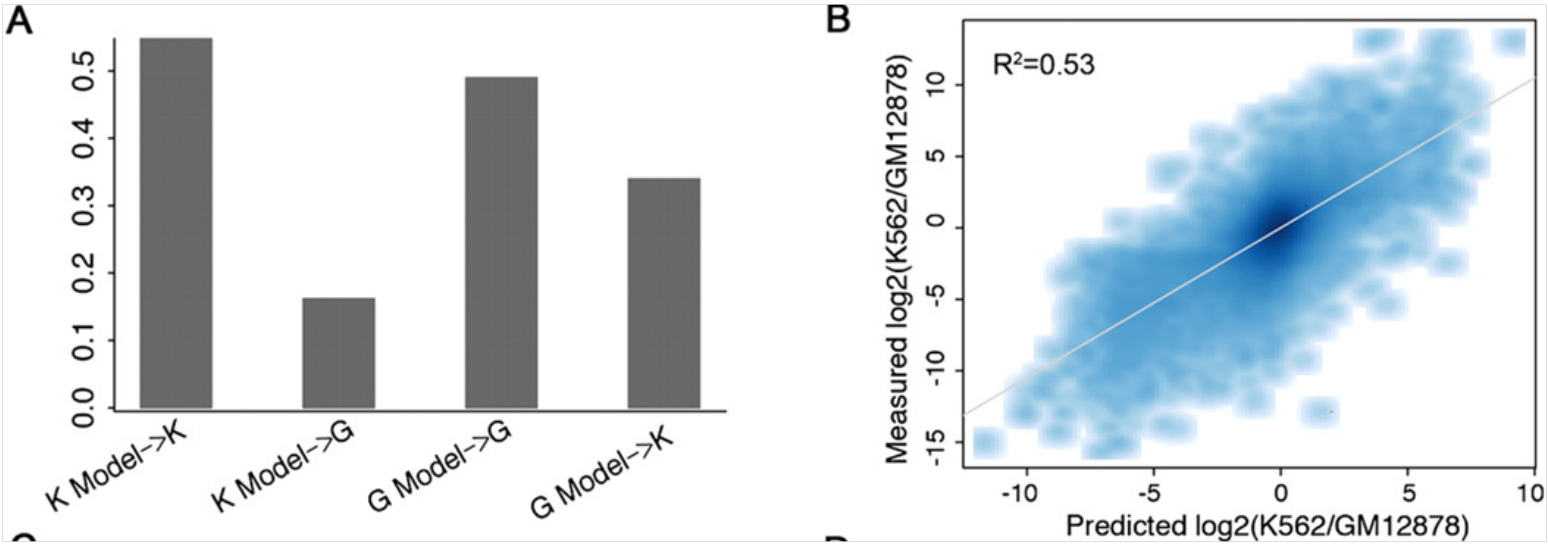

- Cell line specific promoters (fourfold expression difference); 22 TFs
- A: K562 (erythroleukemia) and GM12878 (normal lymphoblastoid) independent models
- B: Using binding differences ($log_2(K562/GM12878)$) to predict expression difference of cell lines.

Cell line specificity of the TF model 2

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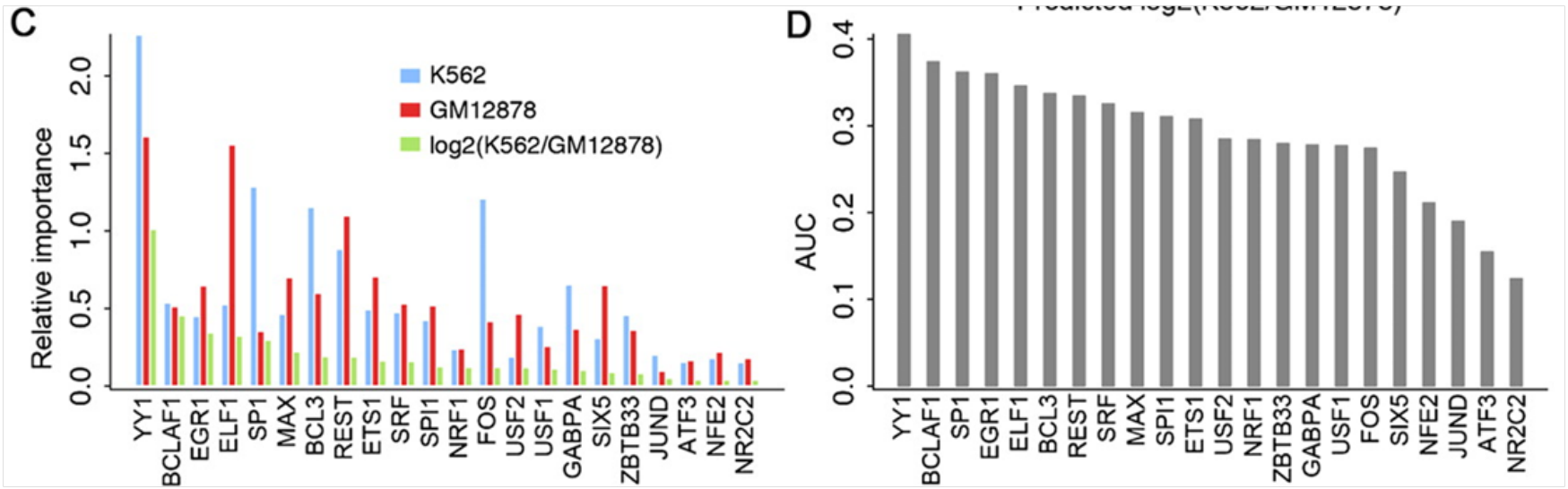
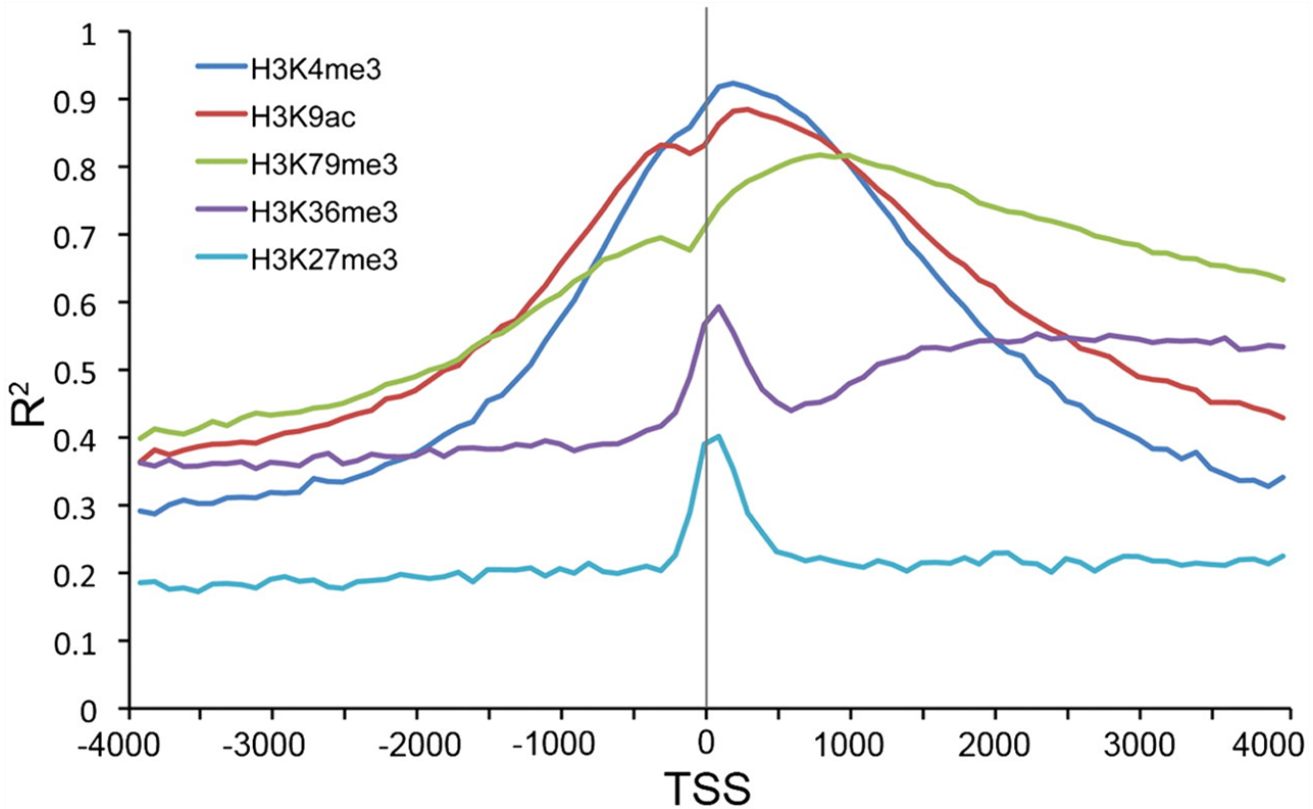


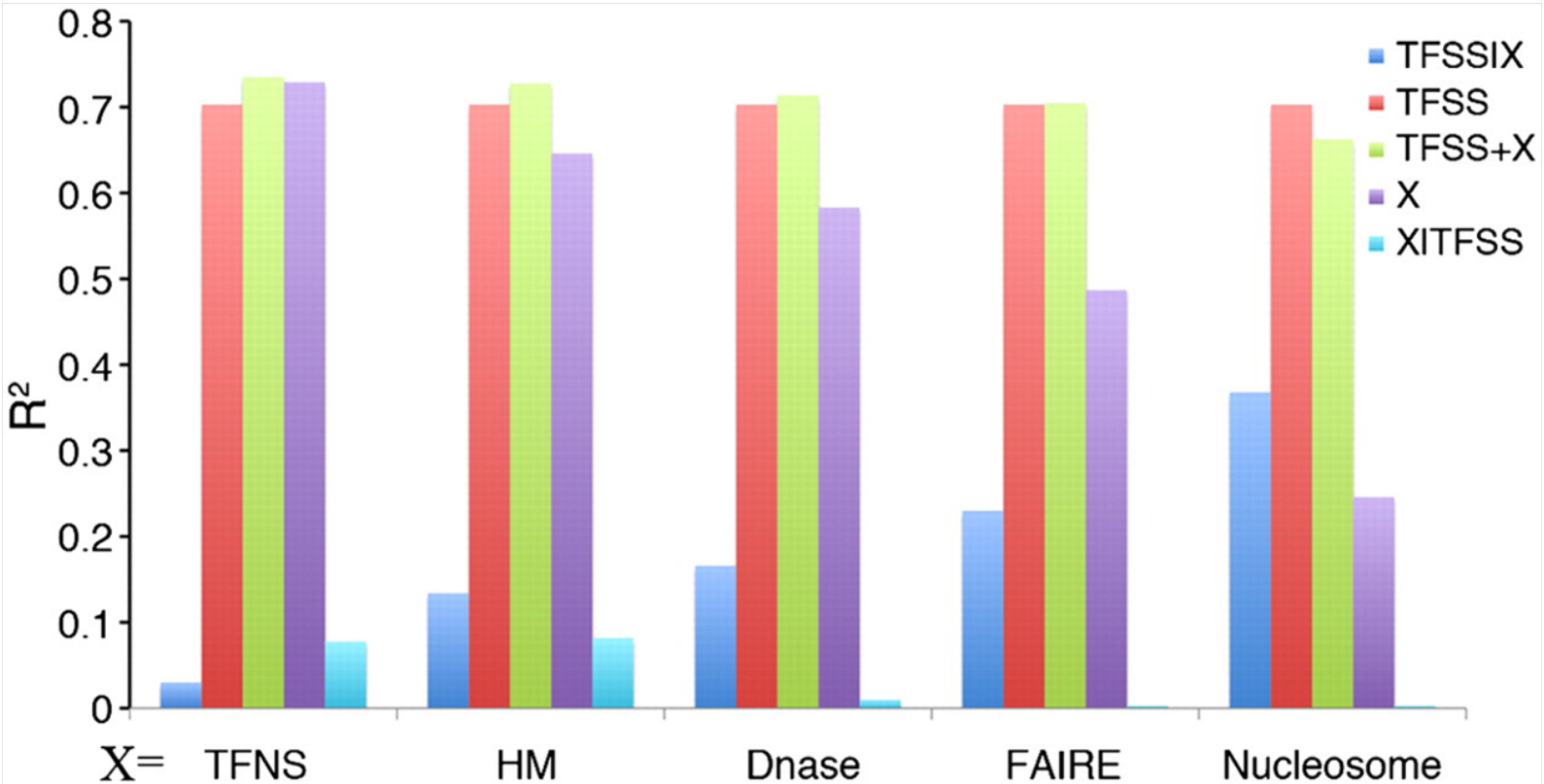
- C: Regression model, RIs of individual TF. Find that TFs with high RIs for differential expression model are TFs with high RIs in both K and G models.
- D: Classification model, using individual TF to classify TSSs in different cell lines. All of the TFs can classify with YY1 the best (AUC=0.86)

The capabilities of TFSS TFs to predict histone modification signals



- Histone modification can be predicted accurately by TF binding signals at TSS region (HsK4me3 $R^2 = 0.85$).
- TSS (-4kb, 4kb) region are divided into 80 bins, each 100bp. Predicting on each bin.

Interplay between TFSS TFs binding and other chromatin features for predicating promoter expression



- Other chromatin structure features: HM (histone modifications), Dnase (DNase hypersensitivity), FAIRE (Formaldehyde Assisted Isolation of Regulatory Elements), and nucleosome occupancy.
- X|TFSS: X $\sim$ y-f(TFSS)
- TFSS+TFNS reaches the best (equals to full model with $R^2 = 0.74$).

Conclusion

Conclusions

- Notable difference in prediction accuracy of expression levels captured by different technologies and protocols
- The expression levels of TSSs with high CpG content are more predictable than those with low CpG content.
- For genes with alternative TSSs, the expression levels of downstream TSSs are more predictable than those of the upstream ones.
- Between two cell lines, the differential expression of TSS can be predicted by the different TF-binding signals.
- TF binding signals and other chromatin features regulate transcription in a coordinated manner.

Regulatory mechanism of TF binding, histone modification, and other chromatin features on gene expression.