Enhancer identification using machine learning enables efficient enhancer RNA targeting with antisense oligonucleotide-based therapeutics

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Motivation

- Antisense oligonucleotides (ASOs) that specifically target functional enhancers and their associated RNAs are being pursued to modulate disease-associated gene expression for a range of therapeutic indications.
- However, identifying functional enhancers and linking them to target genes remains a major challenge.

Approach

Our enhancer-promoter interaction characterization (EPIC) model is a machine learning model for predicting functional enhancer-promoter (E-P) pairs.

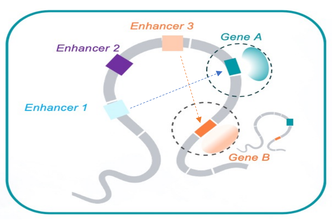
(Gasperini et al., 2019, *Cell*, 176:377-90; Xie et al., 2019, *Cell Reports*, 29: 2570-78)

K562 enhancer epigenomic and chromatin interaction features

K562 CRISPR-based enhancer perturbation screening

EPIC: a machine learning model

Infer



Enhancer 1 – Gene A: Yes
Enhancer 2 – Gene A: No
Enhancer 3 – Gene B: Yes
...

Basic features

- HiChIP.AnchorSize: AnchorSize = 5kb, 10kb, 15kb, or 20kb (n=4)
- Assay.Position.WindowSize, where Assay=ATAC, H3K27ac, H3K4me1, H3K4me3, EP300, CTCF, or Input ChIP; Position = Enh or TSS; WindowSize = 300bp, 500bp, 1kb, 2kb, or 4kb (n=7*2*5=70)
- Genomic distance (n=1)

Feature engineering

APMI = (ATAC.Enh.1kb * EP300.Enh.1kb * H3K4me1.Enh.4kb)$^{1/3}$ * HiChIP.5kb

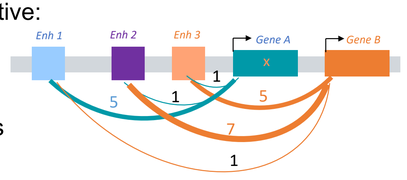
Based on APMI, we engineered a new set of features for quantifying the relative contribution of an enhancer ***e*** to a gene ***g*** from the gene perspective or enhancer perspective:

$$fracGene_{eg} = \frac{APMI_{eg}}{\sum_j APMI_{jg}}$$

where j indexes all the enhancers connected to gene ***g***.

$$fracEnh_{eg} = \frac{APMI_{eg}}{\sum_k APMI_{ek}}$$

where k indexes all the genes connected to enhancer ***e***.



	Enh1 - GeneA	Enh3 -GeneB
fracGene	$\frac{5}{1+1+5} = 0.71$	$\frac{5}{1+5+7} = 0.42$
fracEnh	$\frac{5}{1+5} = 0.83$	$\frac{5}{1+5} = 0.82$

In addition, we combined these features to form new features.

$$fracGmE_{eg} = fracGene_{eg} * fracEnh_{eg}$$

$$fracGpE_{eg} = fracGene_{eg} + fracEnh_{eg}$$

$$apmiGene_{eg} = fracGene_{eg} * APMI_{eg}$$

$$apmiEnh_{eg} = fracEnh_{eg} * APMI_{eg}$$

$$apmiGmE_{eg} = fracGmE_{eg} * APMI_{eg}$$

$$apmiGpE_{eg} = fracGpE_{eg} * APMI_{eg}$$

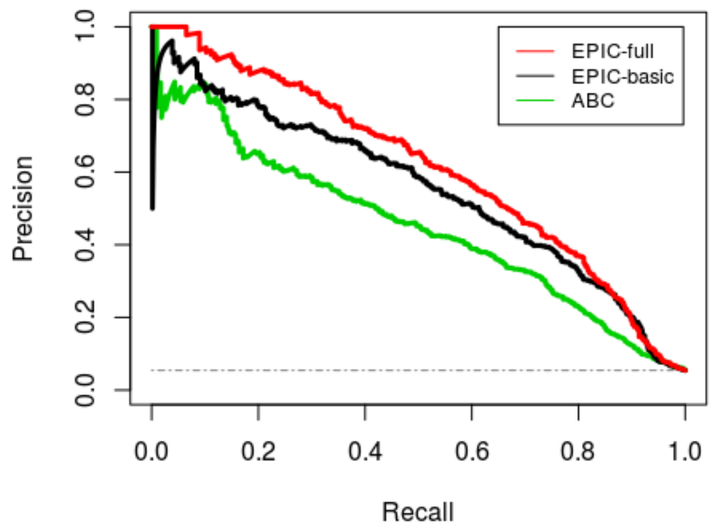
Machine learning model

- Random forest classification model trained on K562 data
- Five-fold cross-validation
- Genetic algorithm for feature selection

Results

EPIC outperforms ABC model in predicting enhancer-promoter pairs (holdout test data)

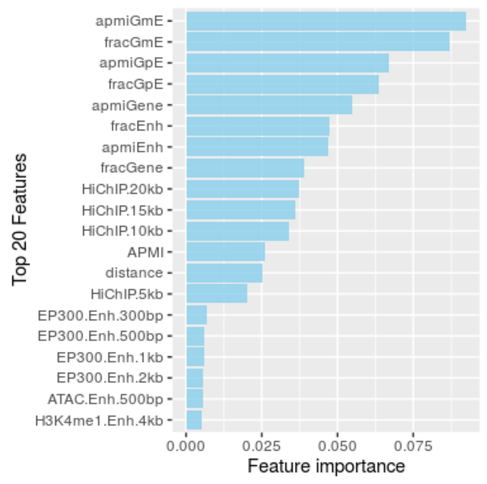
ABC = (ATAC.Enh.500bp * H3K27ac.Enh.500bp)$^{1/2}$ * HiC.5kb (Fulco et al., 2019, *Nat. Genet.*, 51:1664-9)



Model	AUPR	AUROC
EPIC-full	**0.613**	**0.918**
EPIC-basic	0.551	0.912
ABC	0.451	0.885

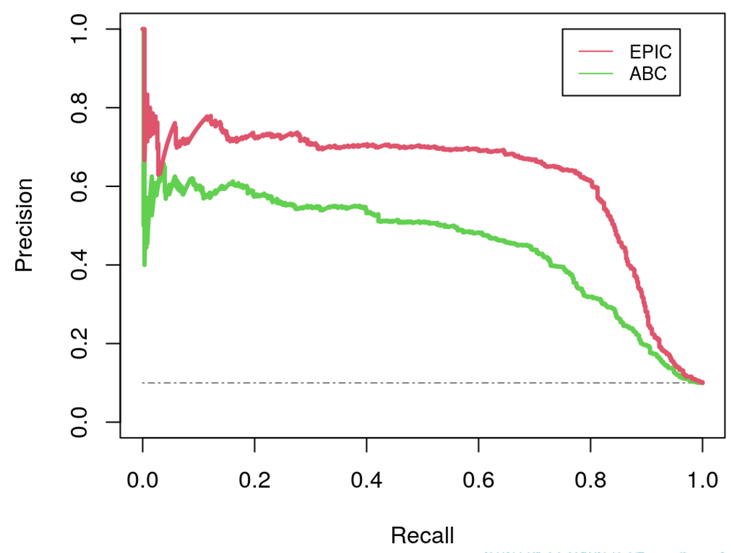
- The area under receiver operating characteristic (AUROC) curve of EPIC-full is significantly higher than that of ABC (p = 7.6e-11) (DeLong et al., 1988, *Biometrics,* 44:837-45).
- The AUROC of EPIC-full is significantly higher than that of EPIC-basic (p = 0.01), demonstrating the value of feature engineering.

Engineered features ranked highest in feature importance



EPIC outperforms ABC model when evaluated with experimental validation in a new cell type

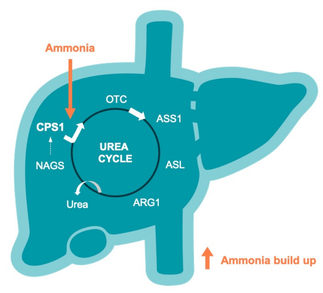
- We conducted a large scale single-cell CRISPRi-based enhancer perturbation screen in HepG2 cells.
- The significant E-P pairs derived from the screen were used to evaluate EPIC and ABC scores.



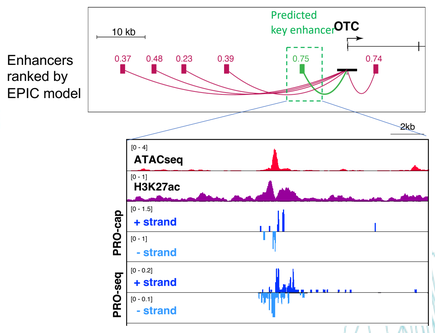
Model	AUPR	AUROC
EPIC	**0.625**	**0.910**
ABC	0.455	0.861

Developing ASOs targeting a regulatory RNA of the human ornithine transcarbamylase (*OTC*) gene

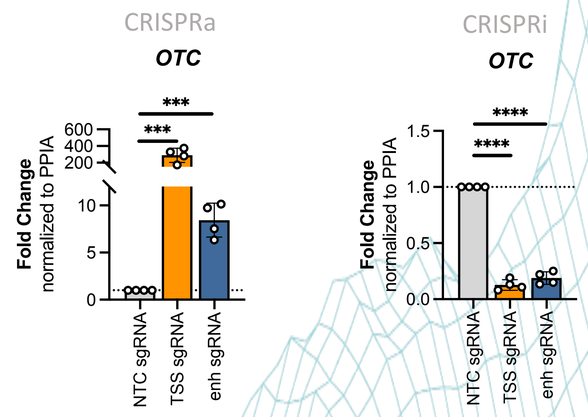
Decreased *OTC* expression results in an inadequate ammonia processing



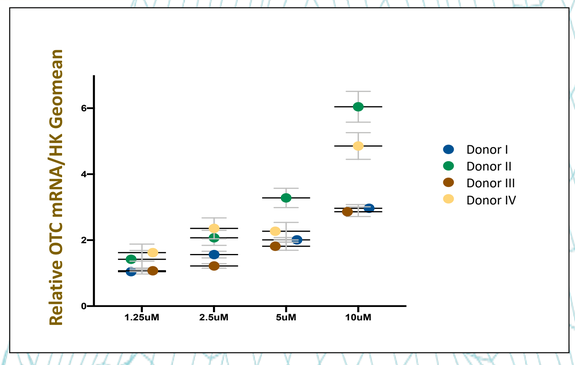
EPIC model was used to identify key *OTC* enhancer in hepatocytes



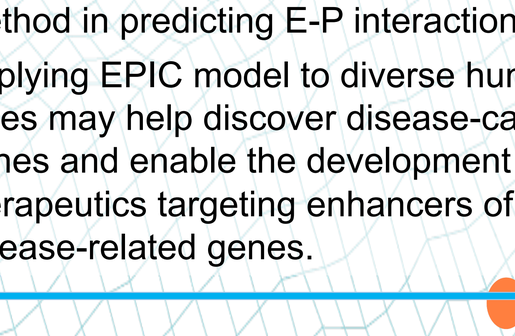
OTC enhancer targeting by CRISPRa or CRISPRi changes *OTC* mRNA levels



ASO targeting *OTC* enhancer RNA (eRNA) shows dose-dependent increase in *OTC* mRNA in hepatocytes from multiple donors



OTC eRNA-targeting ASO increases *OTC* mRNA and ureagenesis in human *OTC*-deficient hepatocytes



OTC-deficient hepatocytes: c.-106C>A variant (Allele ID 480410, late-onset OTC deficiency) - pathogenic (dbSNP: rs749748052) associated with 10-25% of normal OTC activity. *OTC* mRNA and urea levels determined after 6 days of ASO treatment (5 µM ASO). NTC = non-targeting control ASO

Conclusions

- Our EPIC model enables accurate cell-type-specific prediction of functional E-P interactions using epigenomic data.
- EPIC model outperforms an established method in predicting E-P interactions.
- Applying EPIC model to diverse human cell types may help discover disease-causing genes and enable the development of novel therapeutics targeting enhancers of disease-related genes.