Many transcription factors recognize DNA shape

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ENCODE Users Meeting - Stanford, CA

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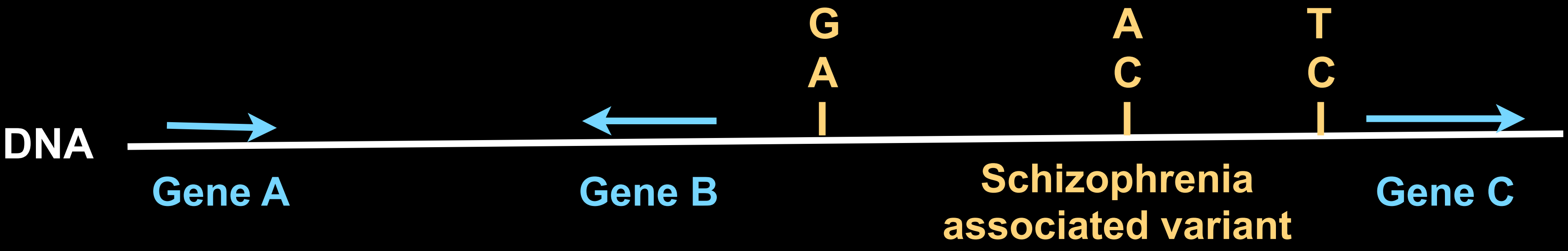
Most disease associated mutations are outside coding regions

Hypothesis 1: Non-coding variants alter transcription factor sequence motifs.



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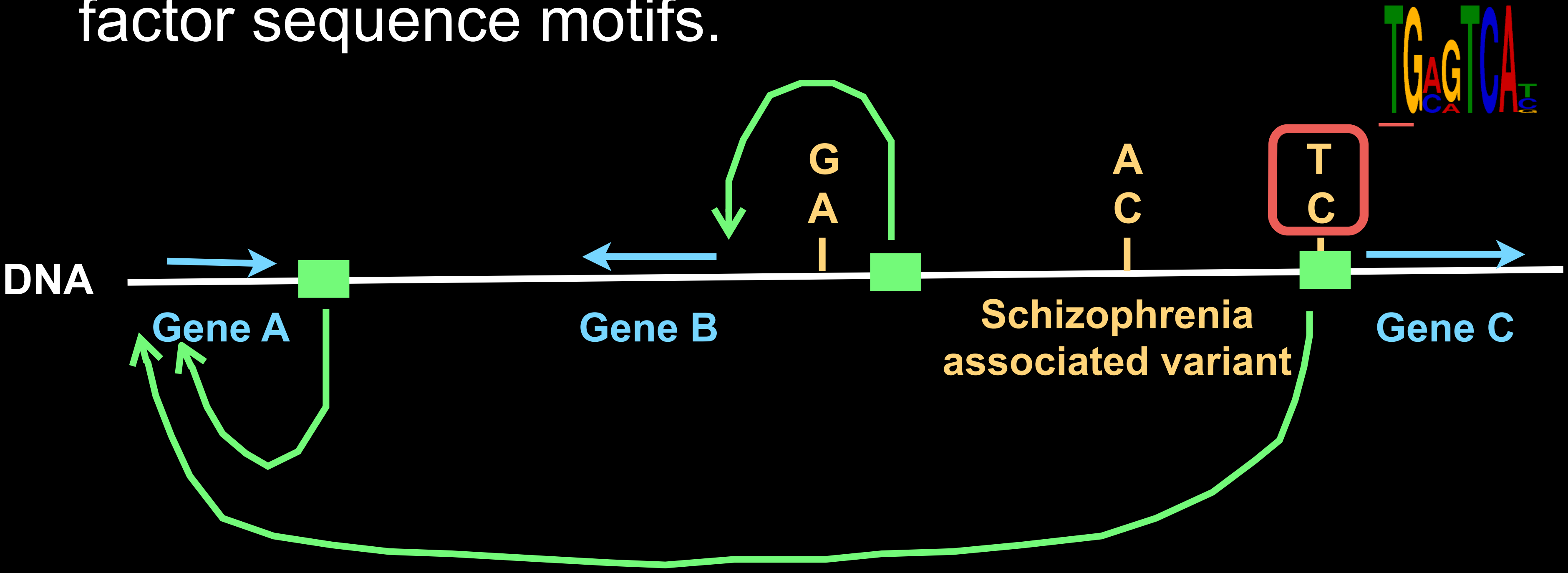
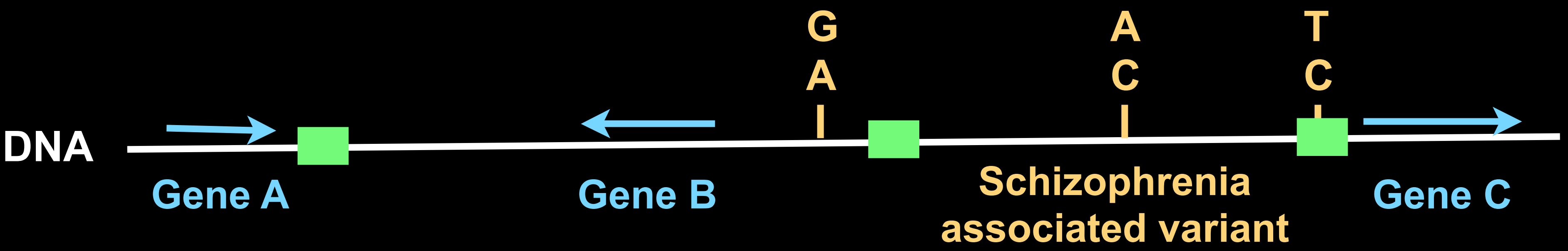


Approach: Map variants to correct pathways by predicting enhancers and their target genes. Score variants for changes in binding affinity.

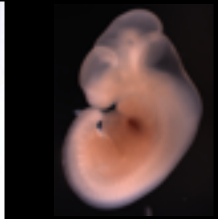
EnhancerFinder distinguishes biologically active enhancers



Training Data

VISTA Enhancers

Yes



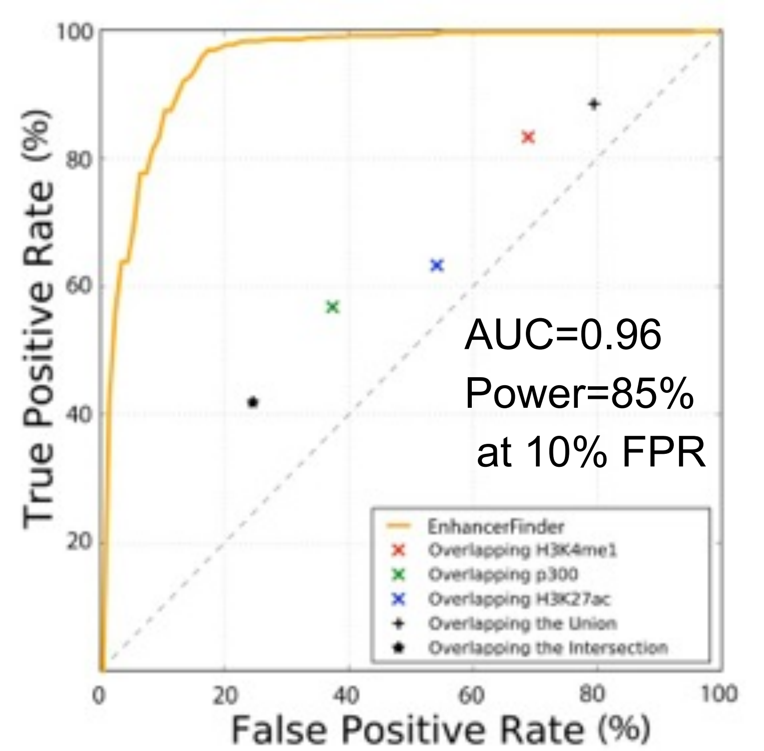
No

Functional Genomics

DNA Sequences

`AAAA,AAAC,AAAG,AAAT,..`

Performance on held out data

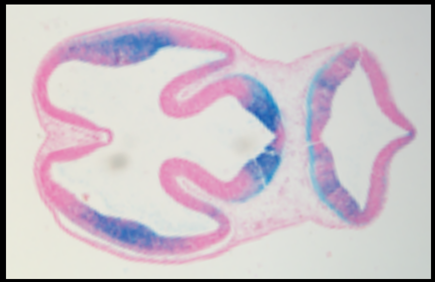


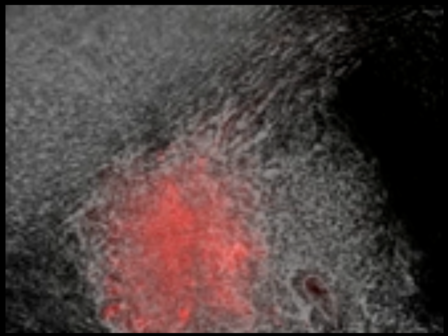
AUC=0.96

Power=85%

at 10% FPR

80% validate in vivo





Erwin et al. (2014)
Capra et al. (2014)

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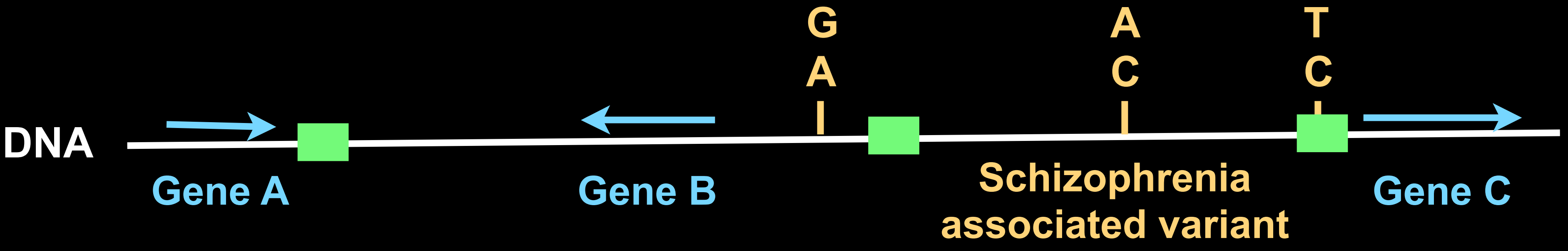

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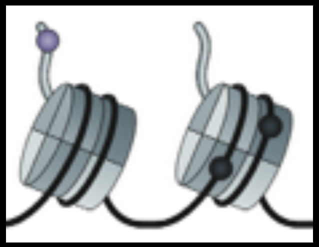

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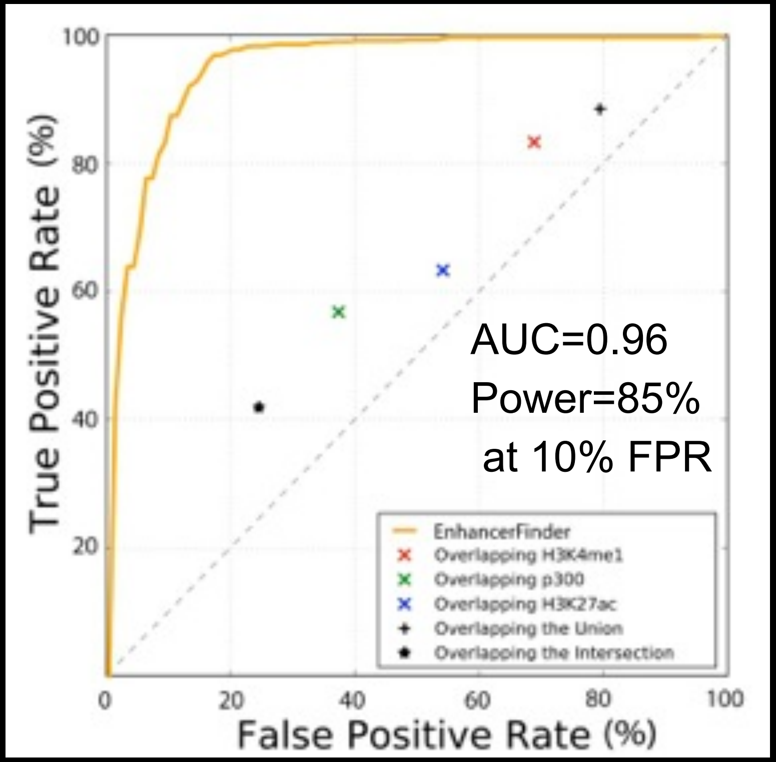

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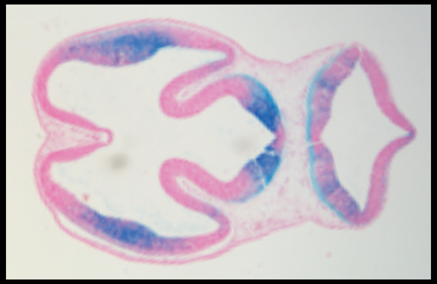

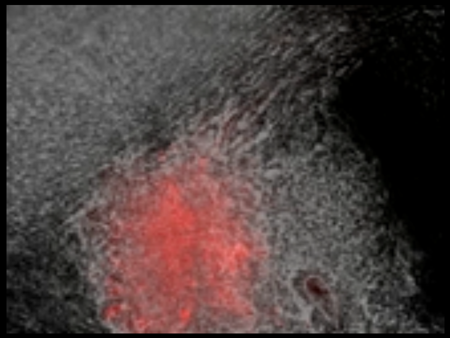

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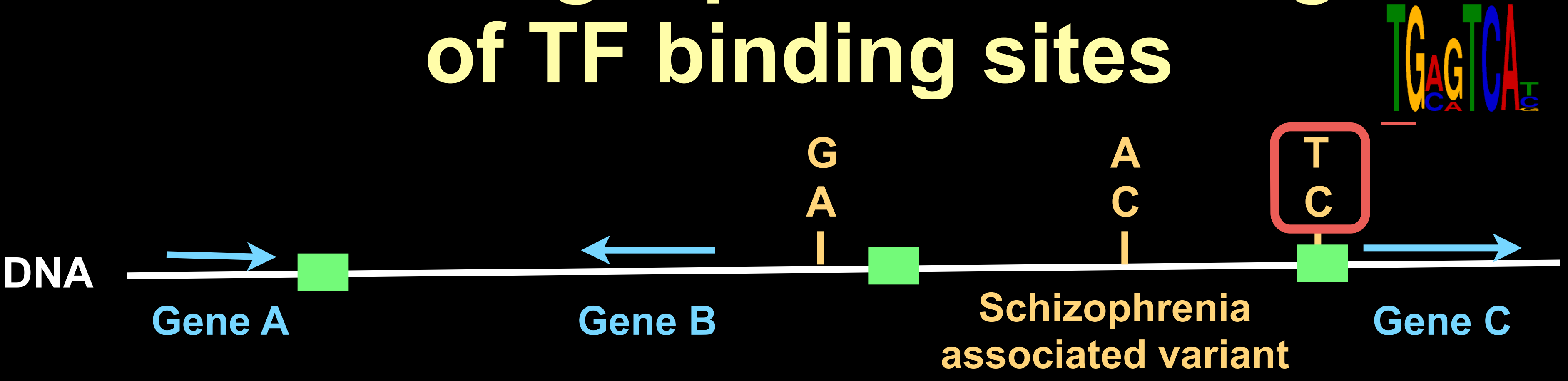
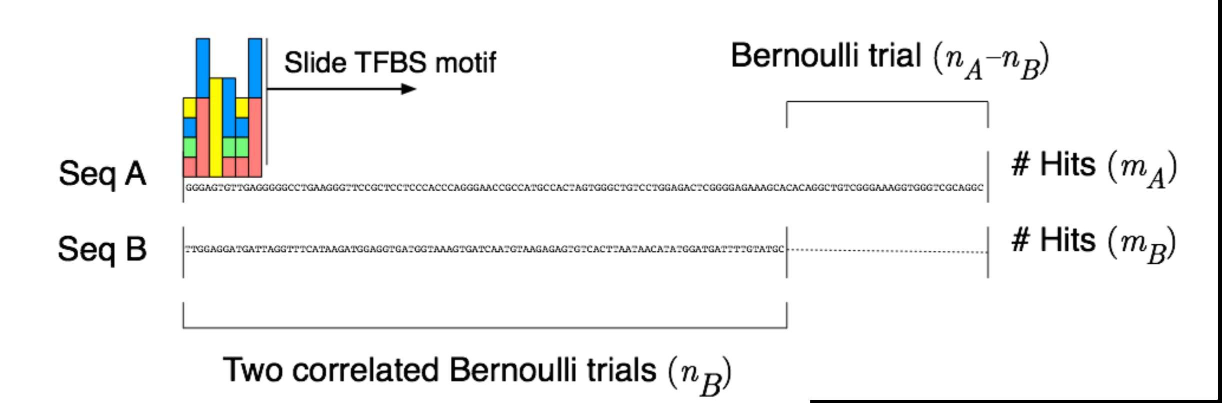
MotifDiverge quantifies loss/gain of TF binding sites



Statistical model for TFBS evolution with turnover

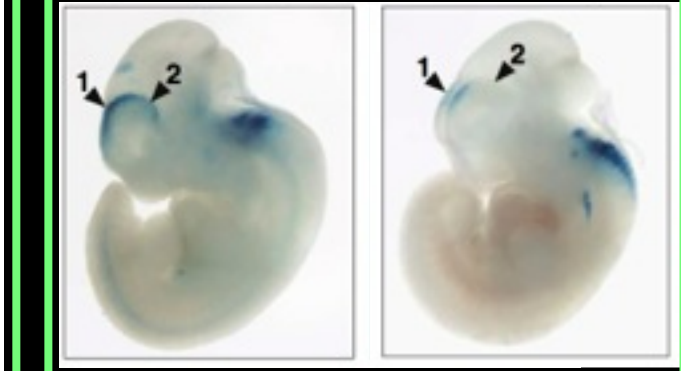

Slide TFBS motif
Bernoulli trial $(n_A - n_B)$
Seq A
GGGAGTGTTGAGGGGGCCTGAAGGGTTCCGCTCCTCCCACCCAGGGAACCGCCATGCCACTAGTGGGCTGTCCTGGAGACTCGGGGAGAAAGCACACAGGCTGTCGGGAAAGGTGGGTCGCAGGC
# Hits (m_A)
Seq B
TTGGAGGATGATTAGGTTTCATAAGATGGAGGTGATGGTAAAGTGATCAATGTAAGAGAGTGTCACTTAATAACATATGGATGATTTTGTATGC
# Hits (m_B)
Two correlated Bernoulli trials (n_B)

$$P(N_{xy} = n_{xy}) = \tag{6}$$
$$\begin{cases} \sum_{j=0}^{k_x - k_y} P_s(N_1 = n_{xy} - j) Bin(N_2 = j) & \text{for} \quad k_x \geq k_y \\ \sum_{j=0}^{k_y - k_x} P_s(N_1 = n_{xy} + j) Bin(N_2 = j) & \text{for} \quad k_x < k_y, \end{cases}$$

P-value for net change in binding

- One or many TFs
- Alignment-free
- Evolutionary model
- Motif specific

Predicts change of function

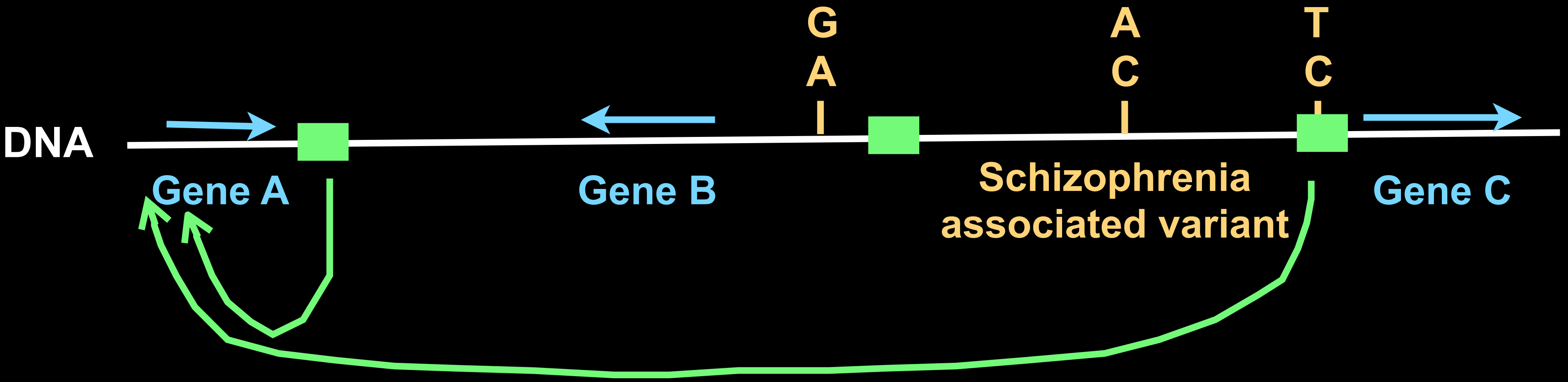


Detects loss/gain of function mutations with high accuracy

- Better than conservation scores
- In vivo and MPRAs in cell lines

Ritter et al. (2010)
Kostka et al. (2015)

TargetFinder maps distal regulatory elements to genes



DNA

Gene A

Gene B

G
A

A
C

T
C

Schizophrenia associated variant

Gene C

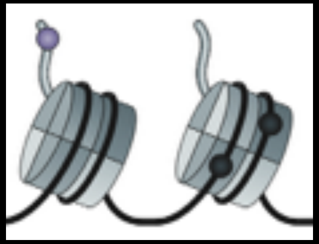
Training Data

Active enhancers

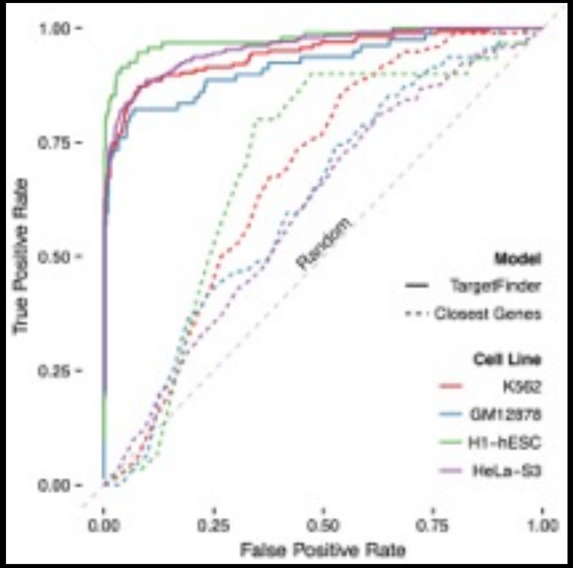
Expressed genes

Hi-C interactions

Functional Genomics



Closest gene usually wrong



Reveals distinct genomic signature of looping DNA

- Heterochromatin on loop
- Cohesin within 6Kb of enhancer and promoter but not mid-loop
- TFs bound with CTCF improve predictions

Whalen et al. (2016)

Summary and Challenges

- Machine-learning on biologically validated enhancers identifies non-coding variants most likely to affect gene regulation and the targeted genes.
  - Massive integration of functional genomics data enables cell type specific predictions
  - Many enhancer-like regions are minimally active and not consistently looping to a target gene

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  - Functional variants outside enhancers

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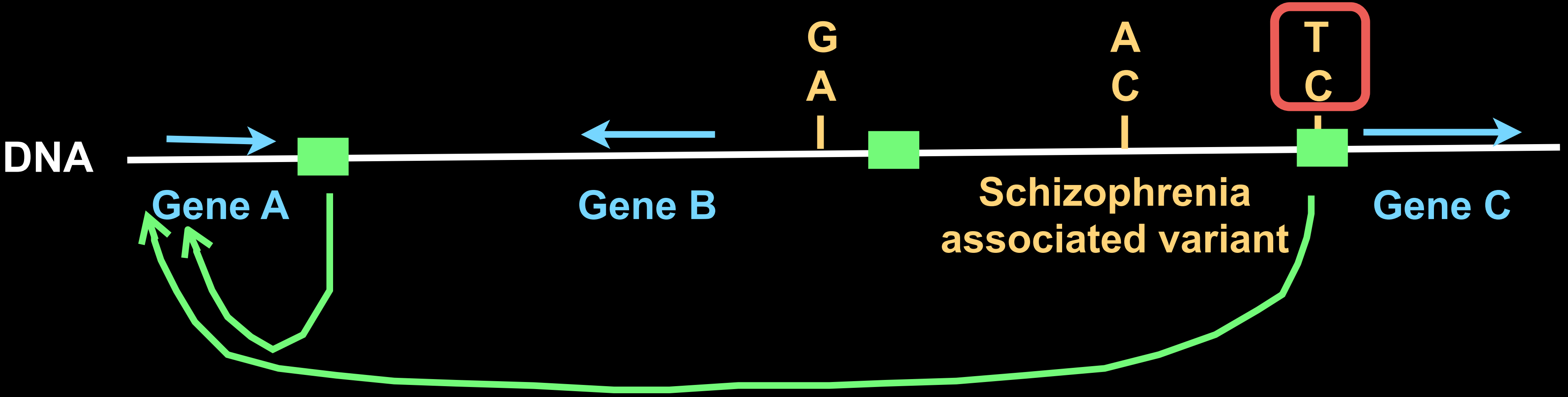

Hypothesis 2: Non-coding variants alter binding sites of structural proteins and chromatin modifiers.

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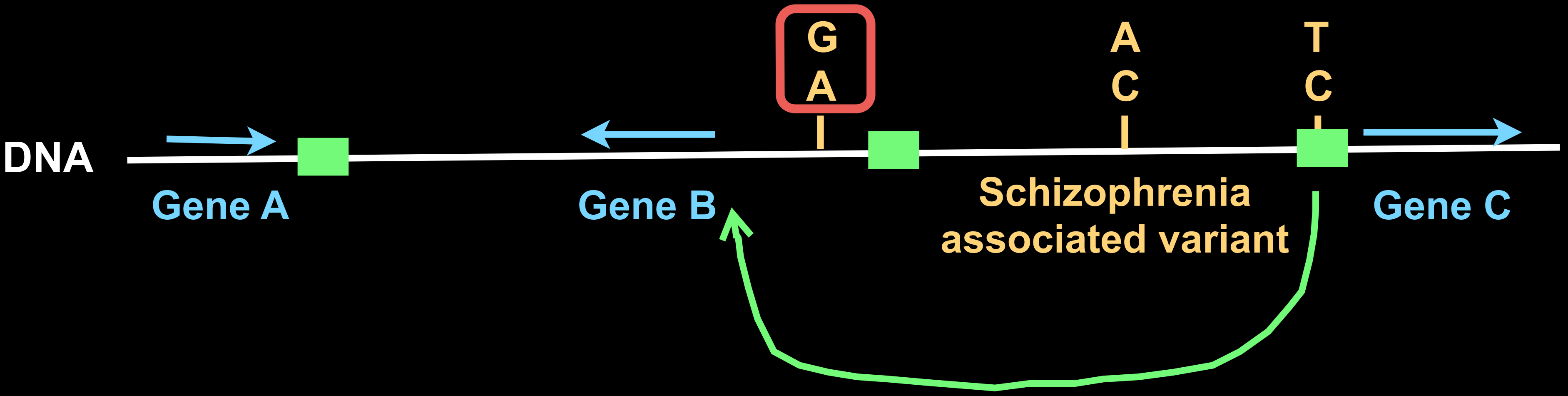

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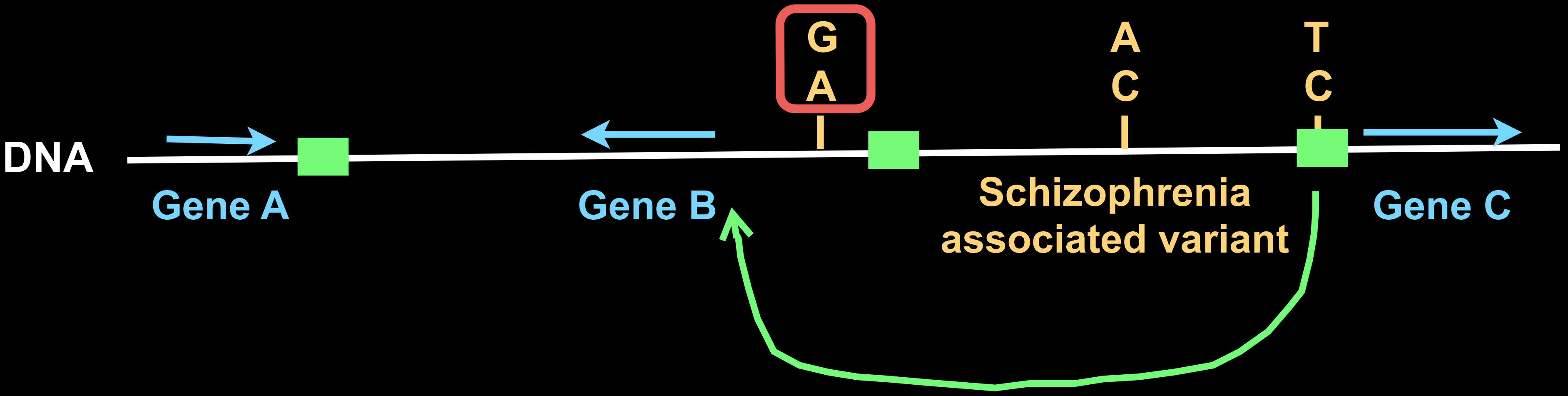

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Approach: CRISPR edit sites identified by TargetFinder, then test chromatin and expression.

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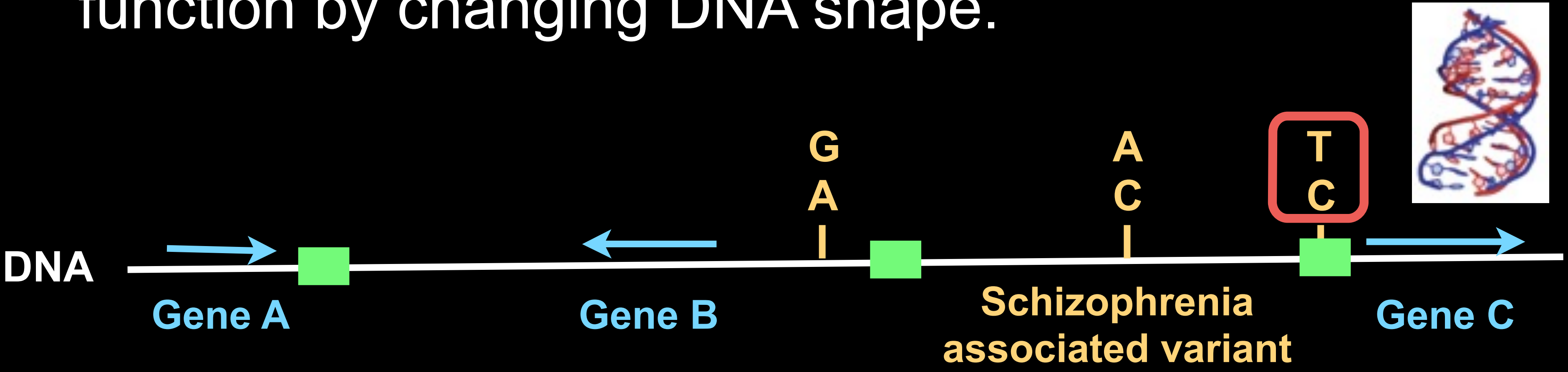
For a typical ENCODE TF 23% of the top 2000 ChIP-seq peaks have no sequence motif (range = 1%-63%)

Many enhancer mutations are outside known or de novo sequence motifs

Hypothesis 3: Non-coding variants alter enhancer function by changing DNA shape.



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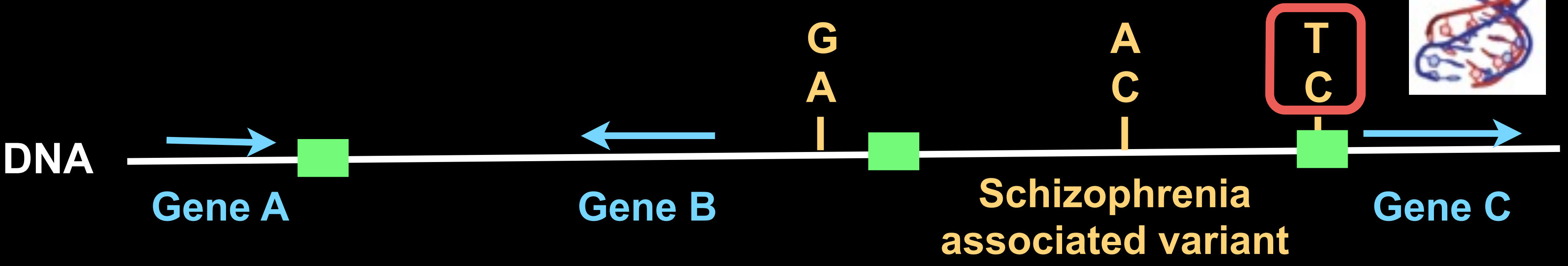

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- DNA shape differentiates similar sequence motifs.
- Distinct sequences can encode same shape.

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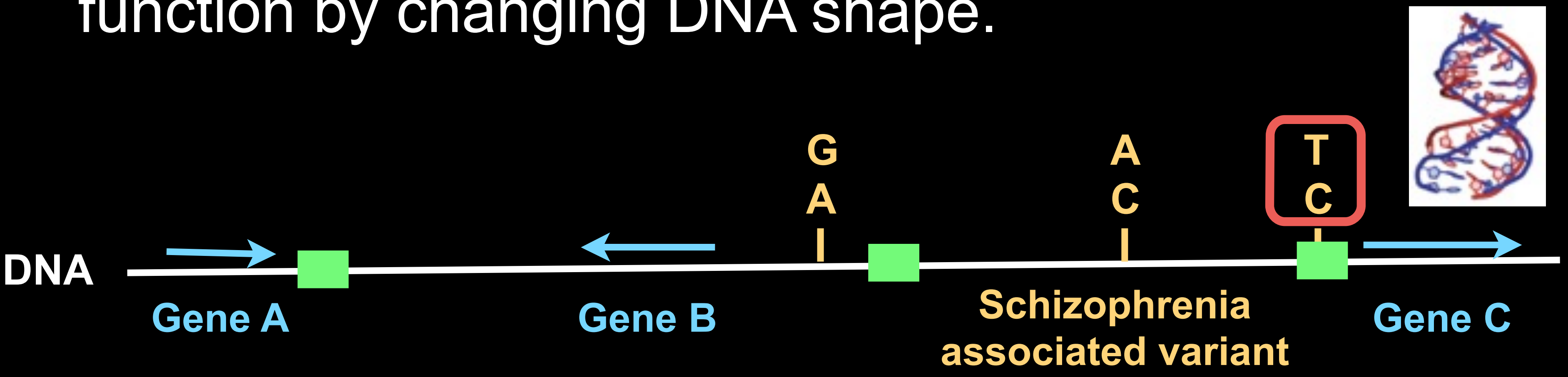

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Approach: Algorithm to learn **shape motifs** de novo for all ENCODE TFs, predict shape motif hits in ChIP-seq peaks, compare to sequence motifs

De novo shape motif discovery

1. Estimate DNA structure: **`DNAshape`** (Zhou et al. 2013)
   - Maps 5-mer sequences to structural features.
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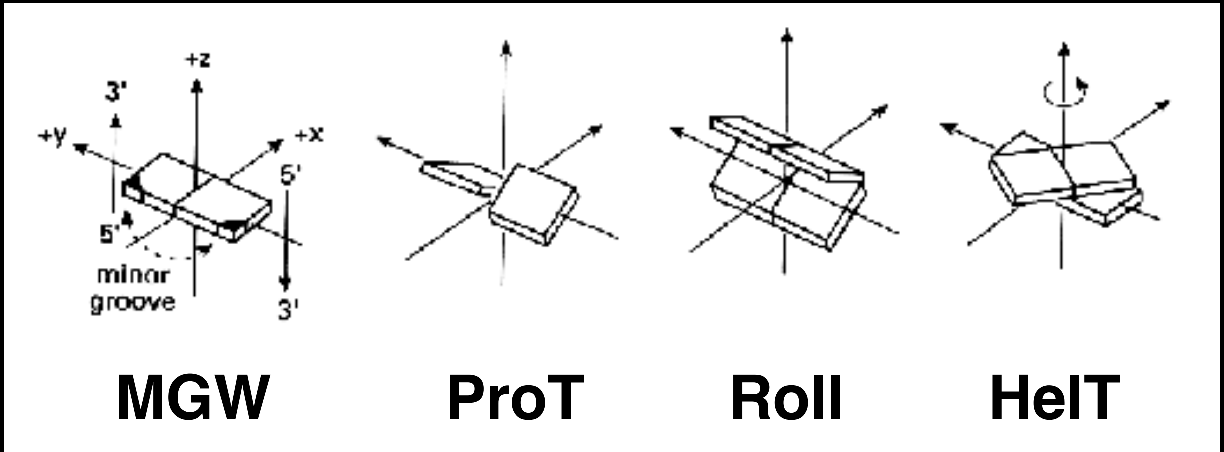

[Figures from Blackburn and Gait, 1996]

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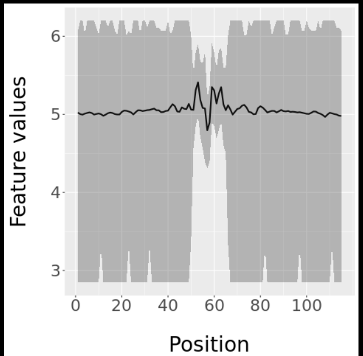

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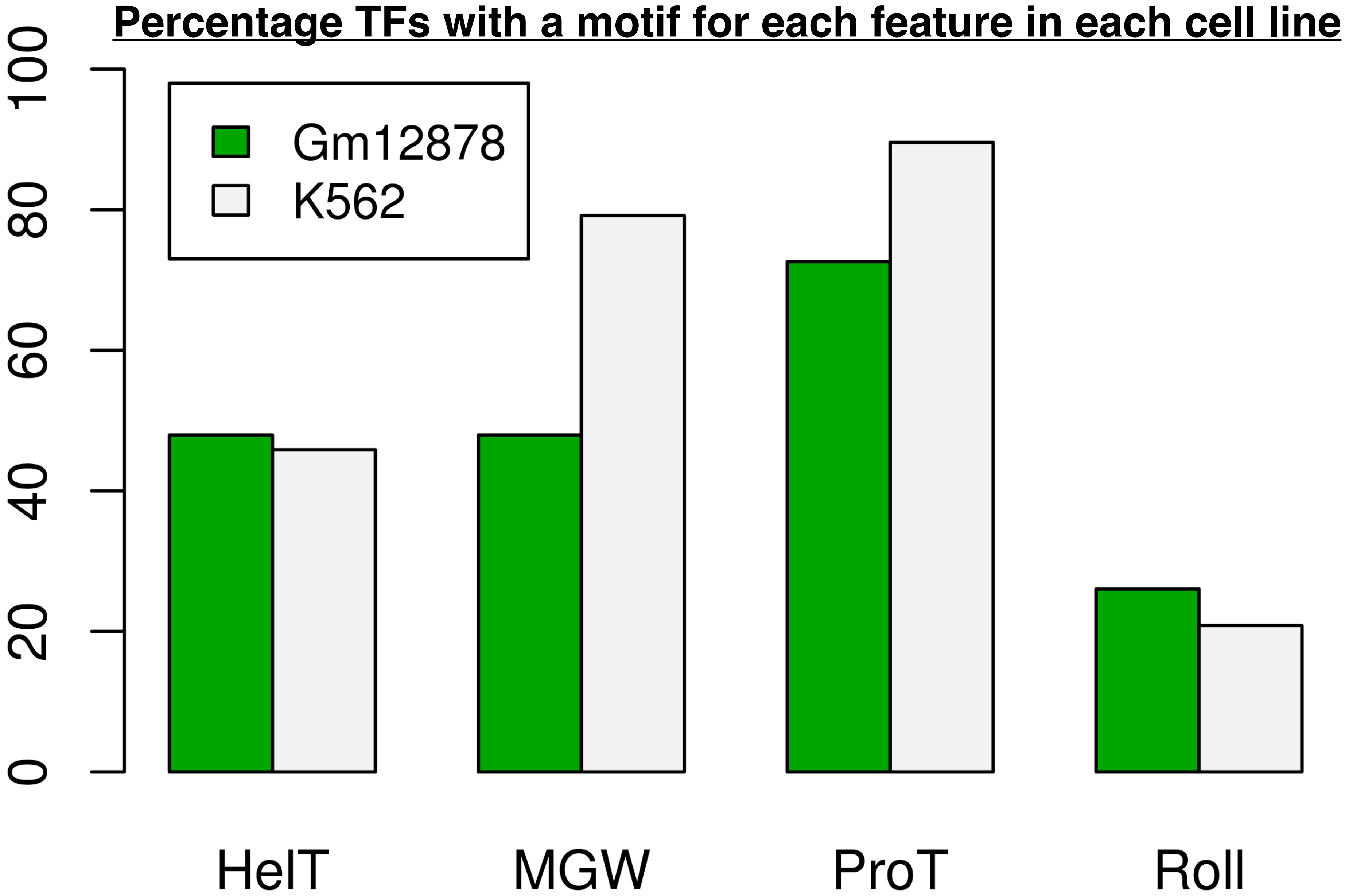
4. Enrichment test: Hypergeometric p-value.

Shape motifs are common



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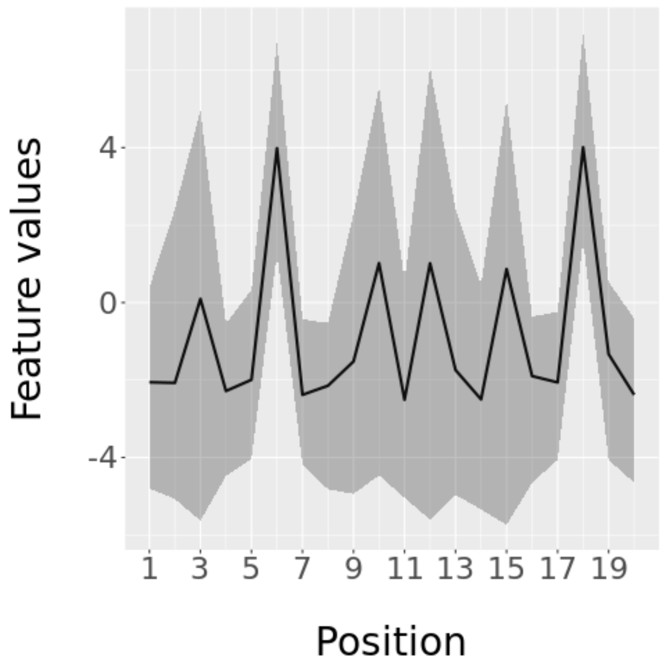
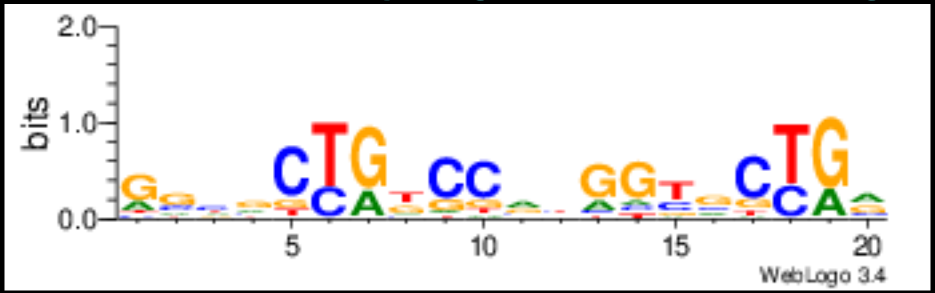
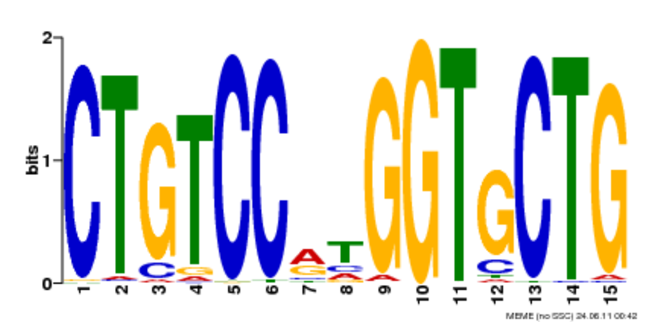


Nrsf Roll motif in K562

Underlying sequence logo





Nrsf FactorBook sequence motif

Shape motifs are complementary

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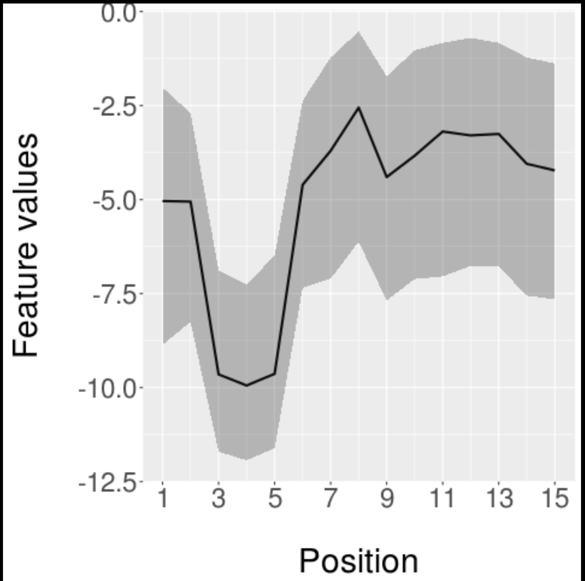
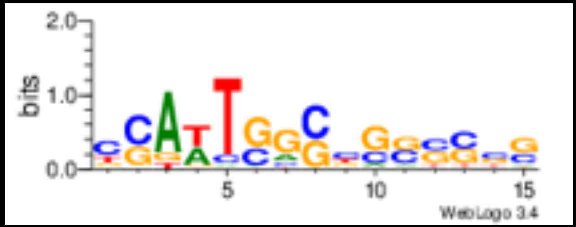
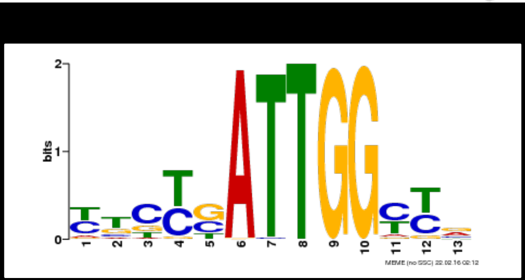


Cfos ProT motif in K562



Underlying sequence



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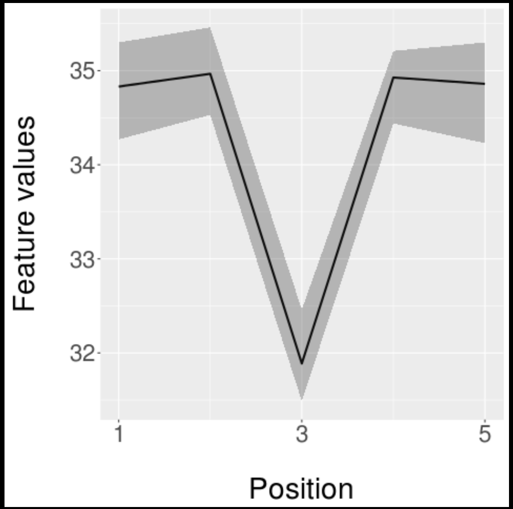
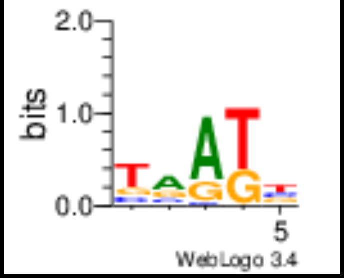
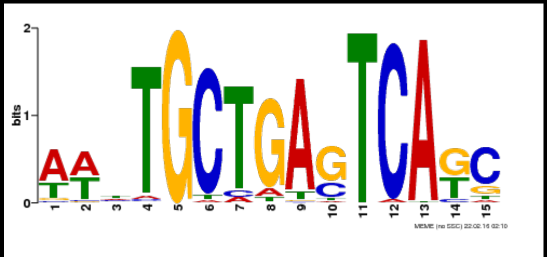


Maff HelT motif in K562



Underlying sequence



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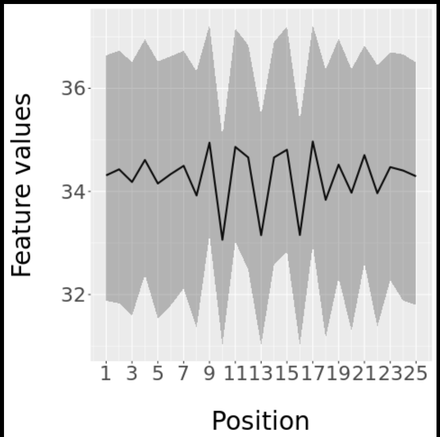
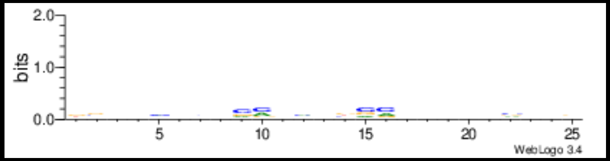
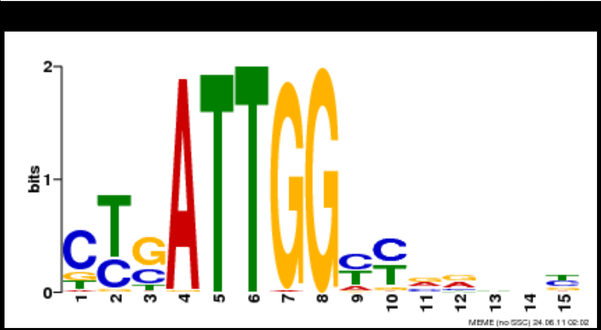


Nfya HelT motif in K562



Underlying sequence



FactorBook sequence motif is 3bp upstream

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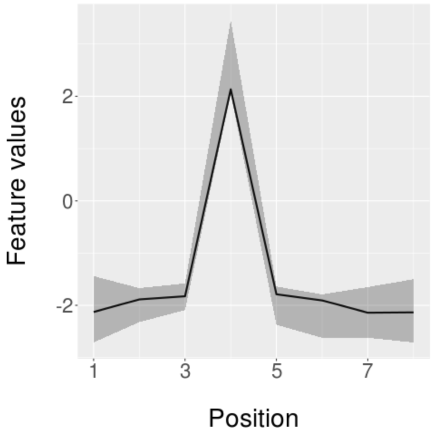
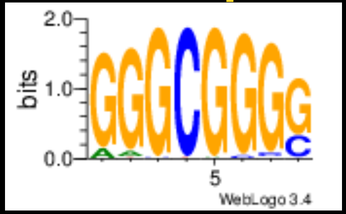


Cfos Roll motif in K562



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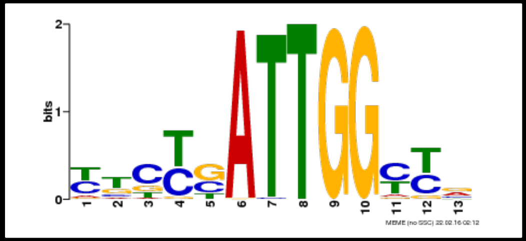

FactorBook sequence motif is 30bp away

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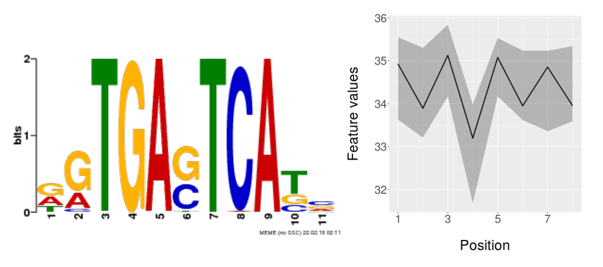
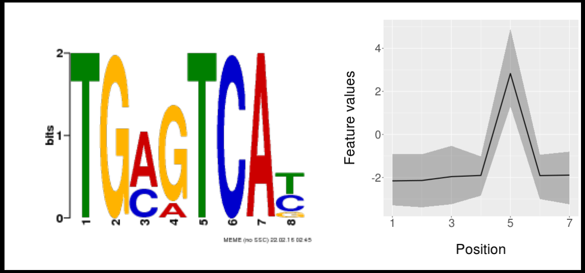
- Shape motifs can differ between TFs with similar sequence motifs and/or the same protein fold.



Fosl1 has a HelT motif



Atf3 has a Roll motif

Ongoing Work

- Hierarchical or mixture model of TF binding with sequence and shape motifs
  - Decompose sequence motifs by shape types
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- Role of shape in ectopic binding of TFs when co-factors are absent [Luna-Zurita et al. 2016]
- Evolutionary modeling of DNA shape
  - Conservation of shape without sequence
  - Scoring SNPs for effects on shape motifs

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