# Interpreting noncoding variants

BMI/CS 776
www.biostat.wisc.edu/bmi776/
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# Goals for lecture

Key concepts

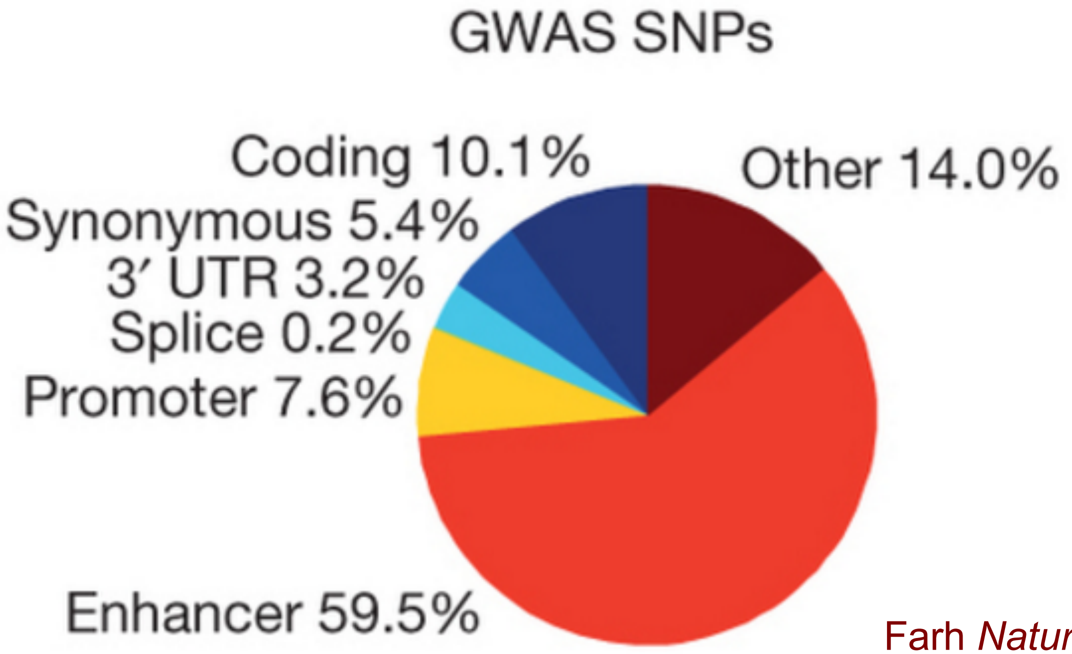
- Mechanisms disrupted by noncoding variants
- Deep learning to predict epigenetic impact of noncoding variants

# GWAS output

- GWAS provides list of SNPs associated with phenotype
- SNP in coding region
  - Link between the protein and the disease?
- SNP in noncoding region
  - What genes are affected?

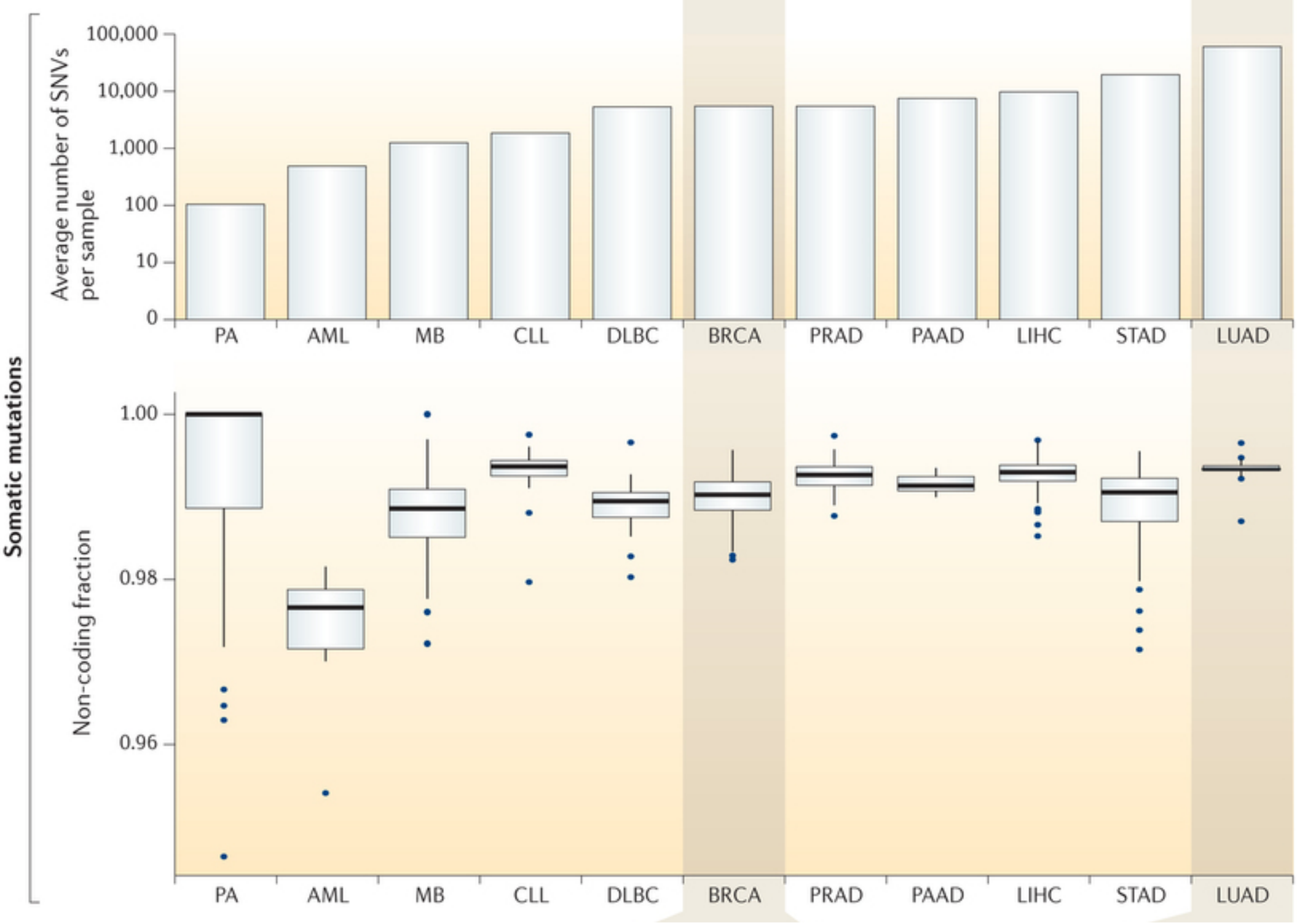
# Noncoding variants common in GWAS

- Meta-analysis of GWAS for 21 autoimmune diseases
  - Rheumatoid arthritis, lupus, multiple sclerosis, etc.
- Method to prioritize candidate causal SNPS
- **90%** of causal variants are noncoding



Farh *Nature* 2015

# Almost all single nucleotide variants in cancer are noncoding



Khurana
*Nature Reviews Genetics*
2016

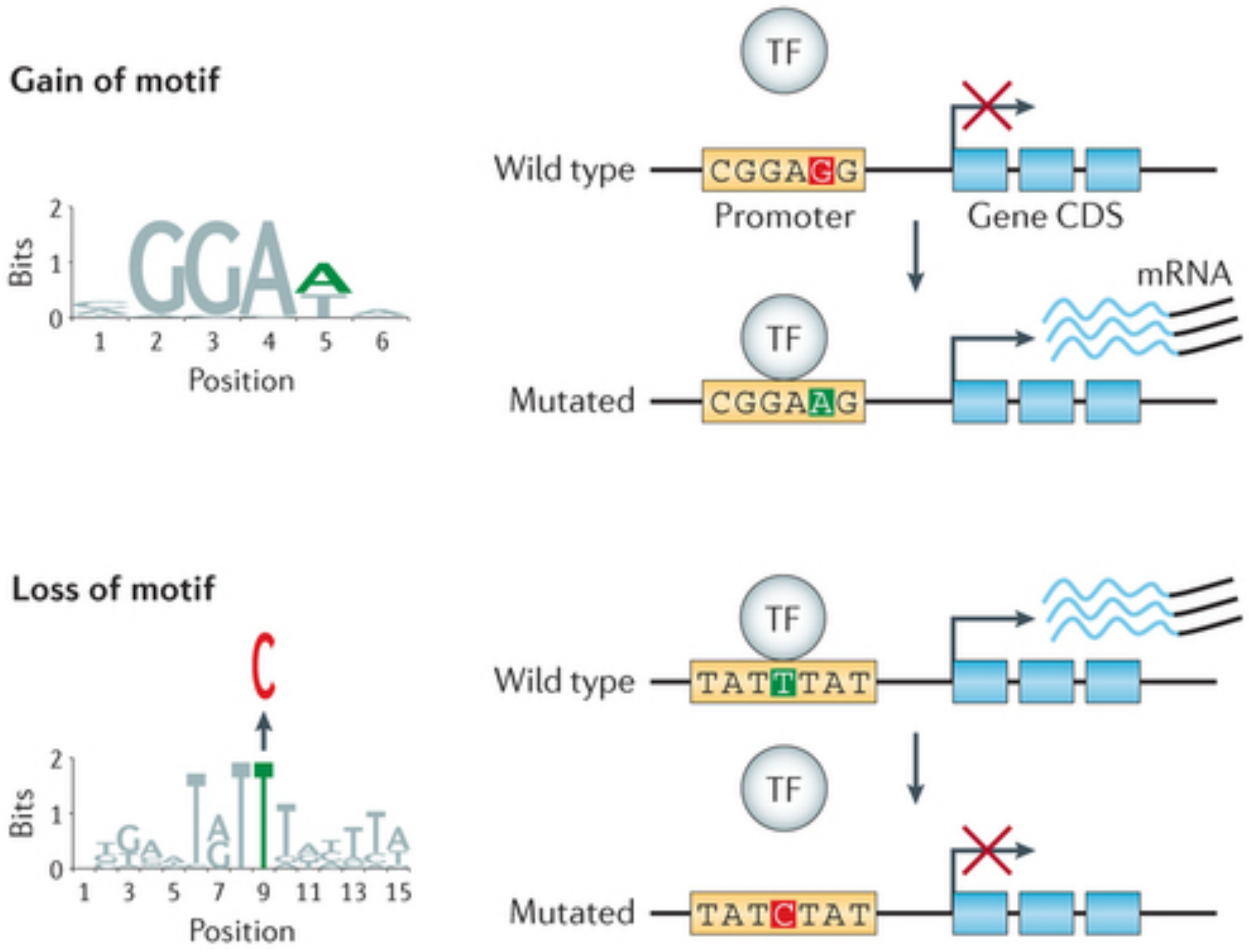
However, very few of these are driver mutations

# Ways a noncoding variant can be functional

- Disrupt DNA sequence motifs
  - Promoters, enhancers
- Disrupt miRNA binding
- Mutations in introns affect splicing
- Indirect effects from the above changes

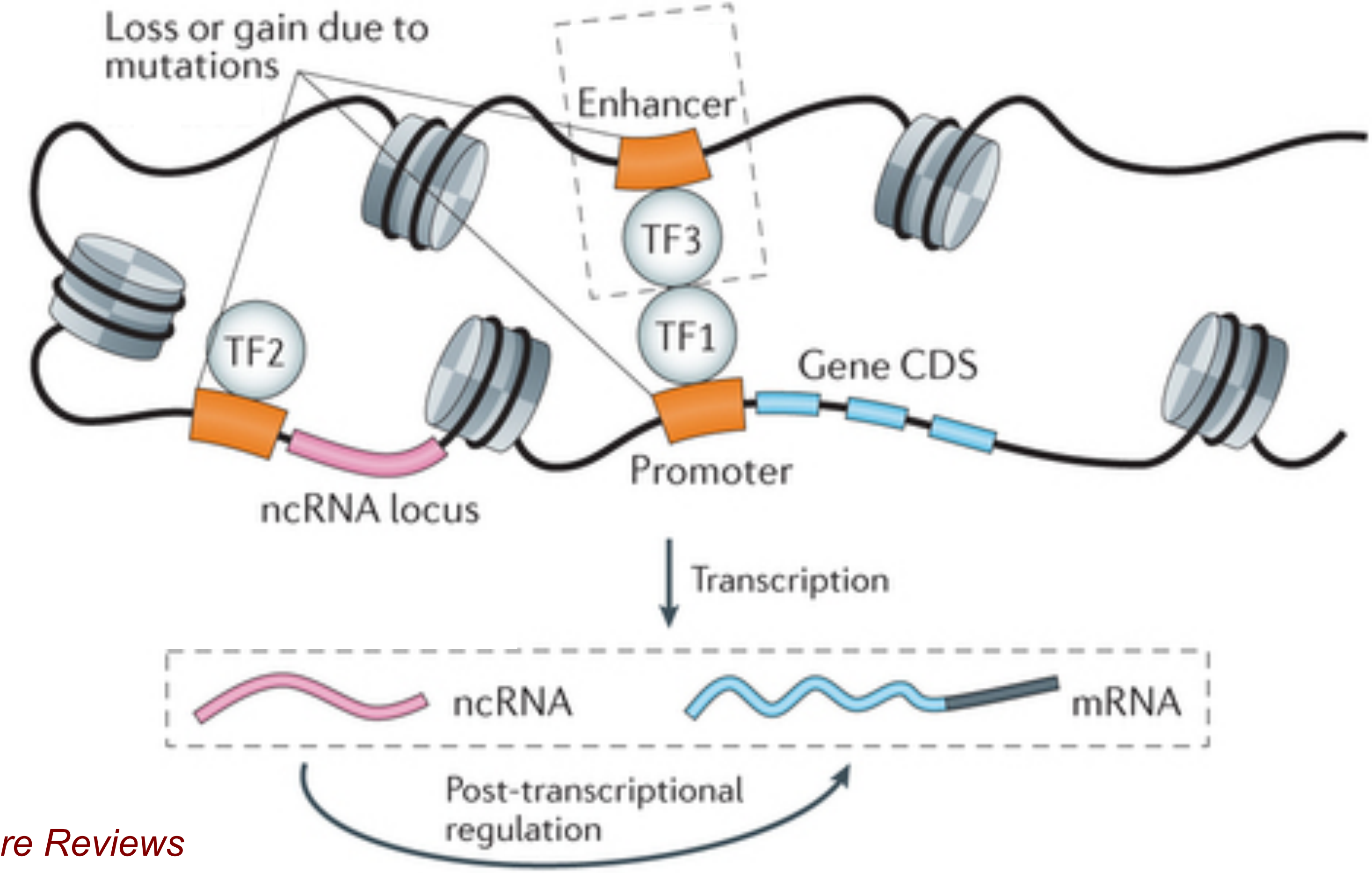
Examples in Ward and Kellis *Nature Biotechnology* 2012

# Variants altering motifs



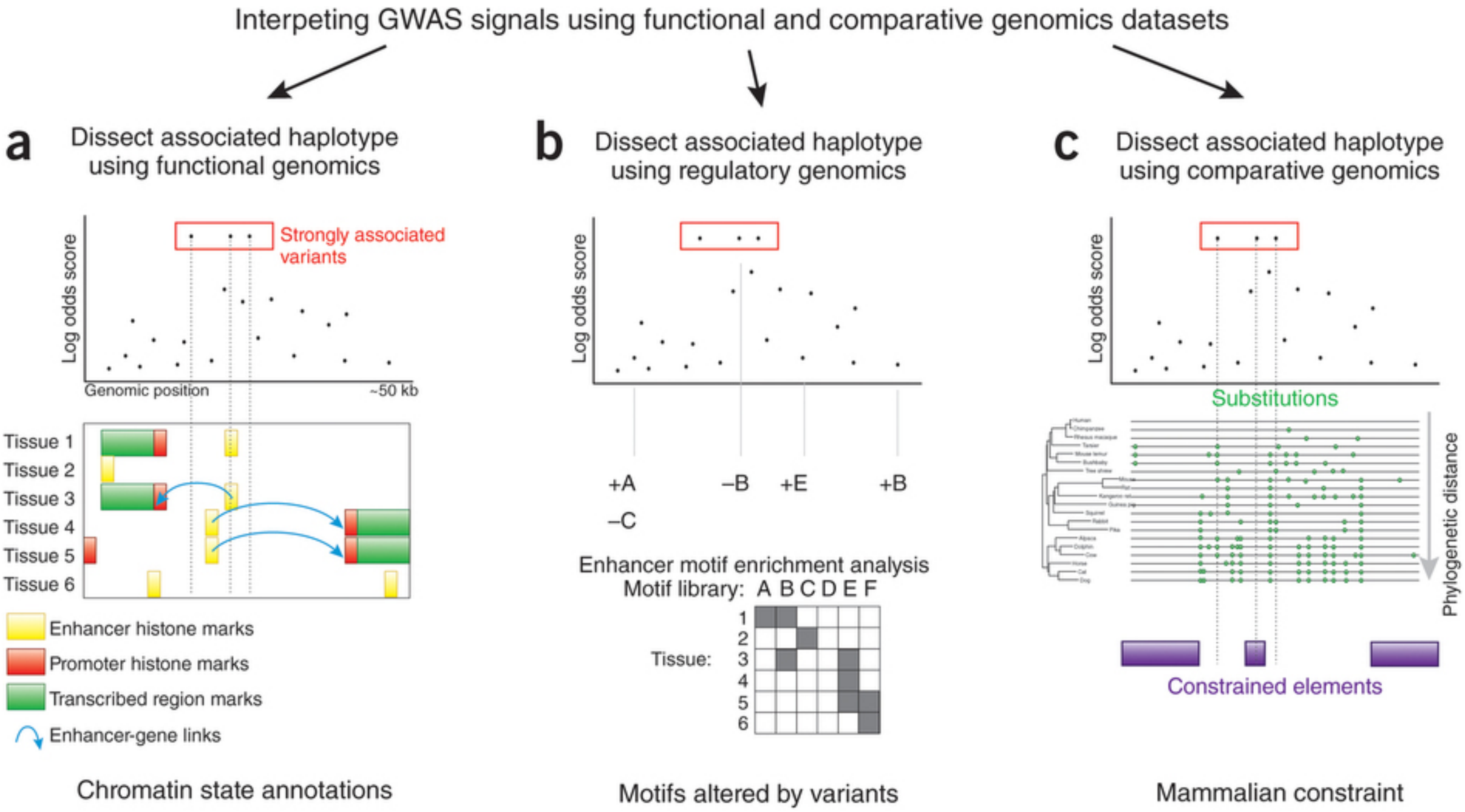
Khurana *Nature Reviews Genetics* 2016

# Variants affect proximal and distal regulators



Khurana *Nature Reviews Genetics* 2016

# Evidence used to prioritize noncoding variants



Ward and Kellis *Nature Biotechnology* 2012

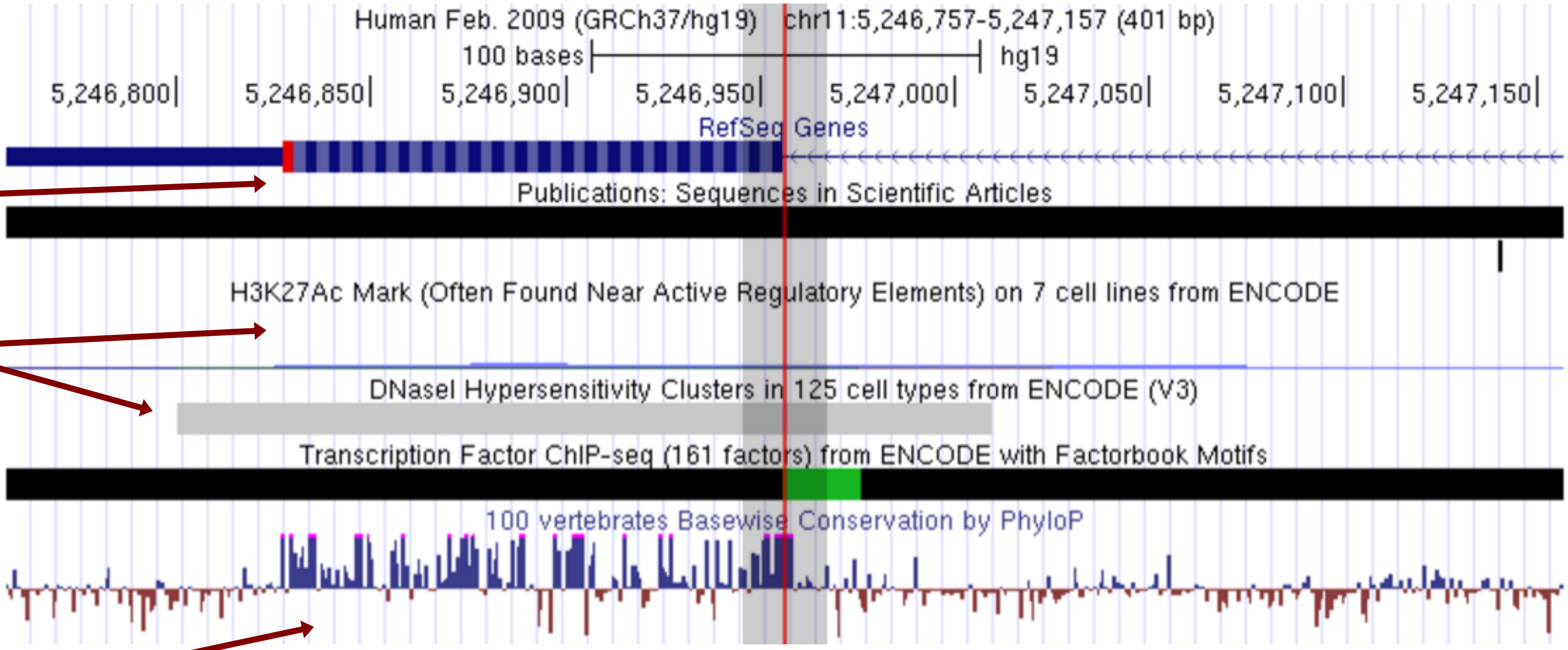
# Visualizing evidence

## Data supporting chr11:5246957 (rs33914668)

Summary of evidence

**Score: 2a**

**Likely to affect binding**


Human Feb. 2009 (GRCh37/hg19) chr11:5,246,757-5,247,157 (401 bp)

RefSeq Genes

Publications: Sequences in Scientific Articles

H3K27Ac Mark (Often Found Near Active Regulatory Elements) on 7 cell lines from ENCODE

DNaseI Hypersensitivity Clusters in 125 cell types from ENCODE (V3)

Transcription Factor ChIP-seq (161 factors) from ENCODE with Factorbook Motifs

100 vertebrates Basewise Conservation by PhyloP

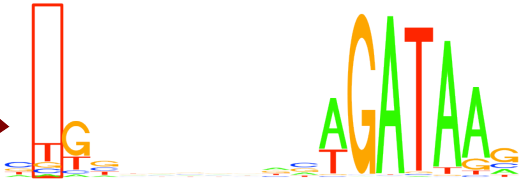
Genes

Epigenetic annotations

Conservation

Boyle *Genome Research* 2012

Affected motifs



# Combined Annotation–Dependent Depletion (CADD)

- Example of an algorithm that integrates multiple types of evidence into a single score
  - Conservation
  - Epigenetic information
  - Protein function scores for coding variants
- Train support vector machine on simulated and observed variants
- Variants present in simulation but not observed are likely deleterious

Kircher *Nature Genetics* 2014

# Prioritizing variants with epigenetics summary

\+ Disrupted regulatory elements one of the best understood effects of noncoding SNPs

\+ Make use of extensive epigenetic datasets

\+ Similar strategies have actually worked

  - rs1421085 in *FTO* region and obesity
  - Claussnitzer *New England Journal of Medicine 2015*

\- Epigenetic data at a genomic position is often in the presence of the reference allele

  - Don’t have measurements for the SNP allele

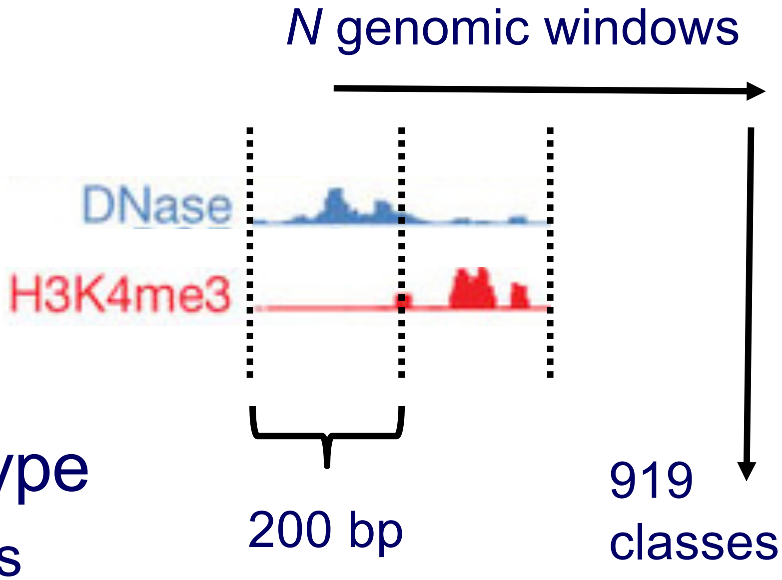
# DeepSEA

- Given:
  - A sequence variant and surrounding sequence context
- Do:
  - Predict TF binding, DNase hypersensitivity, and histone modifications in multiple cell and tissue types
  - Predict variant functionality

Zhou and Troyanskaya *Nature Methods* 2015

# Classifier input and output

- Output
  - 200 bp windows of genome
  - Label 1 if window contains peak
  - Label for each epigenetic data type
    - Multiple types of epigenetic features
    - Multiple types of cells and tissues



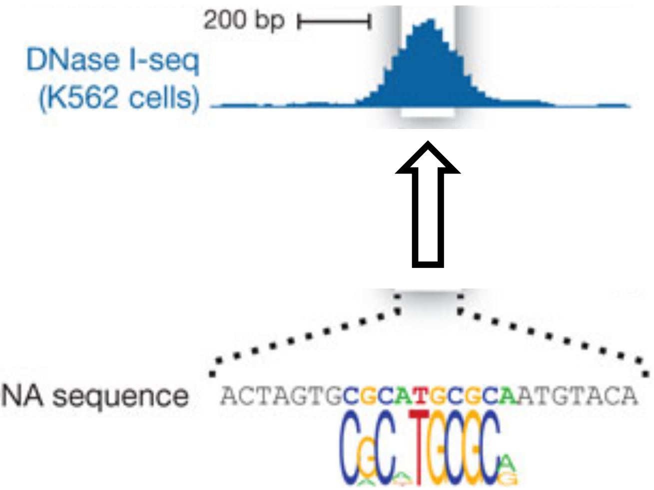
Roadmap Epigenomics Consortium *Nature* 2015

- Input: 1000 bp DNA sequence centered at window

$$x_i = \begin{array}{lcc:cccc:c} \texttt{index} & \texttt{1} & \ldots & \texttt{401} & \texttt{402} & \texttt{403} & \ldots & \texttt{1000} \\ \texttt{A} & 0 & & 1 & 0 & 0 & & 0 \\ \texttt{C} & 0 & & 0 & 0 & 0 & & 1 \\ \texttt{G} & 1 & & 0 & 1 & 1 & & 0 \\ \texttt{T} & 0 & & 0 & 0 & 0 & & 0 \end{array}$$

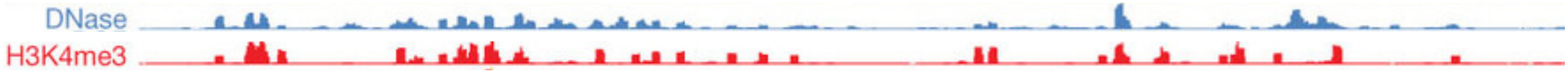
# Desired properties for epigenomic classifier

- Learn preferences of DNA-binding proteins
  - Locally: “motifs” and other simple sequence patterns
  - Sequence context: “*cis*-regulatory modules”
- Support nonlinear decision boundaries



Neph *Nature* 2012

- Multiple, related prediction tasks

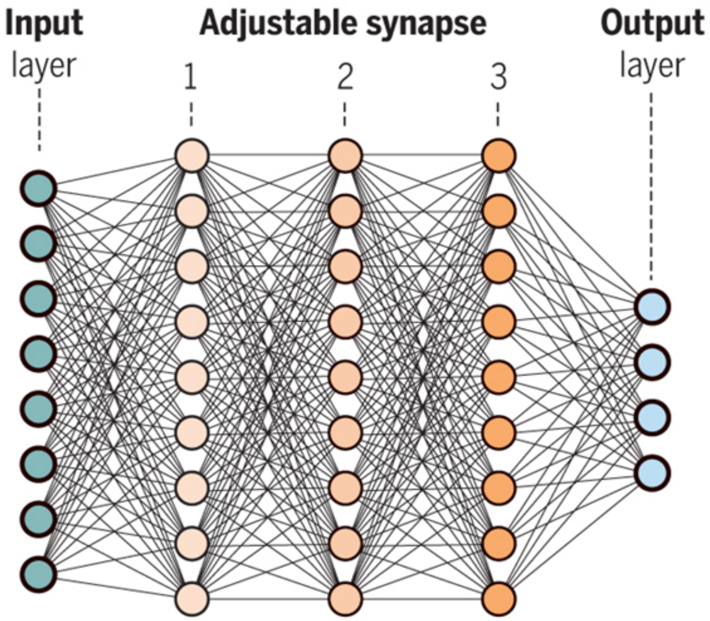


Roadmap Epigenomics Consortium *Nature* 2015

# Neuroscience to artificial intelligence

## Brain circuitry and learning

A major open question is whether the highly simplified structures of current network models compared with cortical circuits are sufficient to capture the full range of human-like learning and cognition.



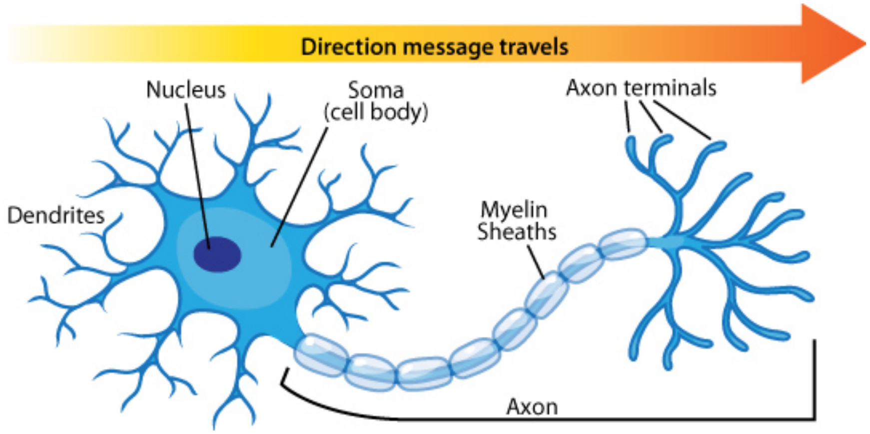
**Complex neural network**
Connectivity in cortical networks includes rich sets of connections, including local and long-range lateral connectivity, and top-down connections from high to low levels of the hierarchy.

**Informed AI network**
Biological innate connectivity patterns provide mechanisms that guide human cognitive learning. Discovering similar mechanisms, by machine learning or by mimicking the human brain, may prove crucial for future artificial systems with human-like cognitive abilities.

Shimon Ullman, Science 15 Feb 2019:
Vol. 363, Issue 6428, pp. 692-693

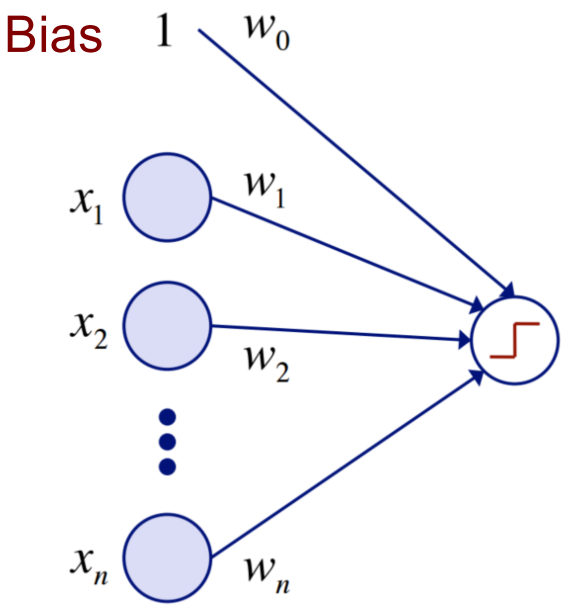
# Perceptron

- Inspired by neuron



Ask a biologist

- Simple binary classifier
  - Linear decision boundary



$$o = \begin{cases} 1 & \text{if } w_0 + \sum_{i=1}^{n} w_i x_i > 0 \\ 0 & \text{otherwise} \end{cases}$$

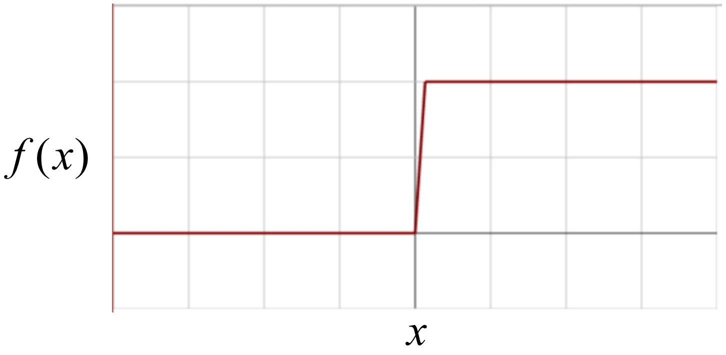
Mark Craven CS 760 slides

$$x_{1,A} \ldots x_{1000,T}$$

# Activation function

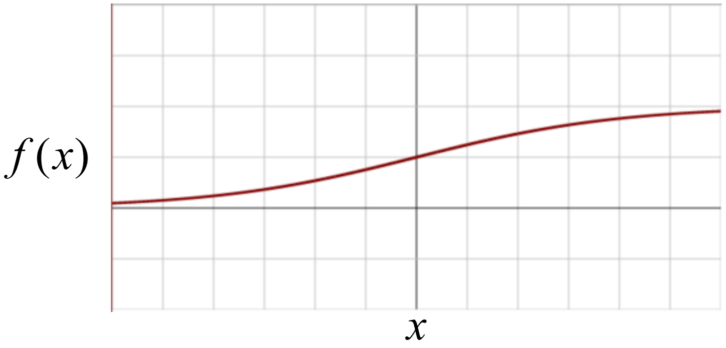
- What makes the neuron “fire”?
  - Step function

$$f(x)=\begin{cases}0 & \text{if } x<0\\ 1 & \text{if } x\geq 0\end{cases}$$



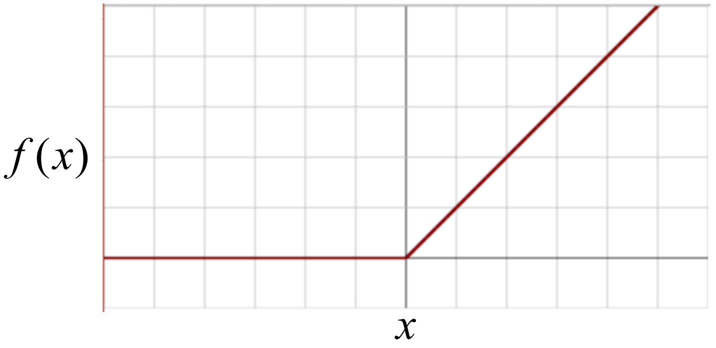
  - Sigmoid function

$$f(x)=\frac{1}{1+e^{-x}}$$



  - Rectified linear unit (ReLU)

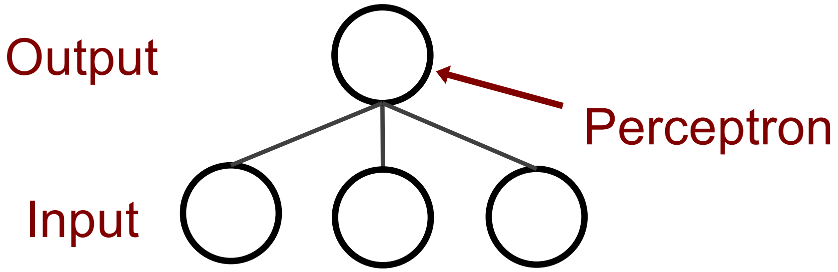
$$f(x)=\max(0,x)$$



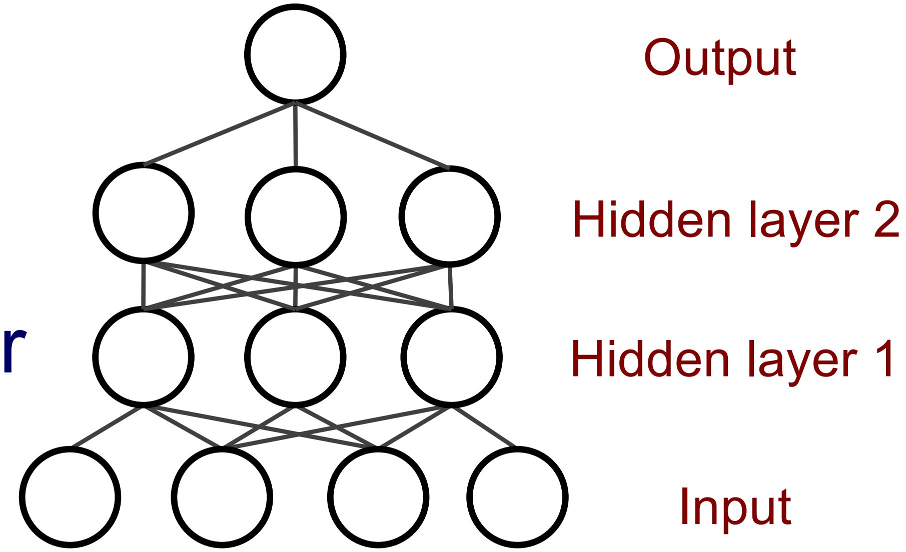
Images from Wikipedia: Activation function

# Neural networks

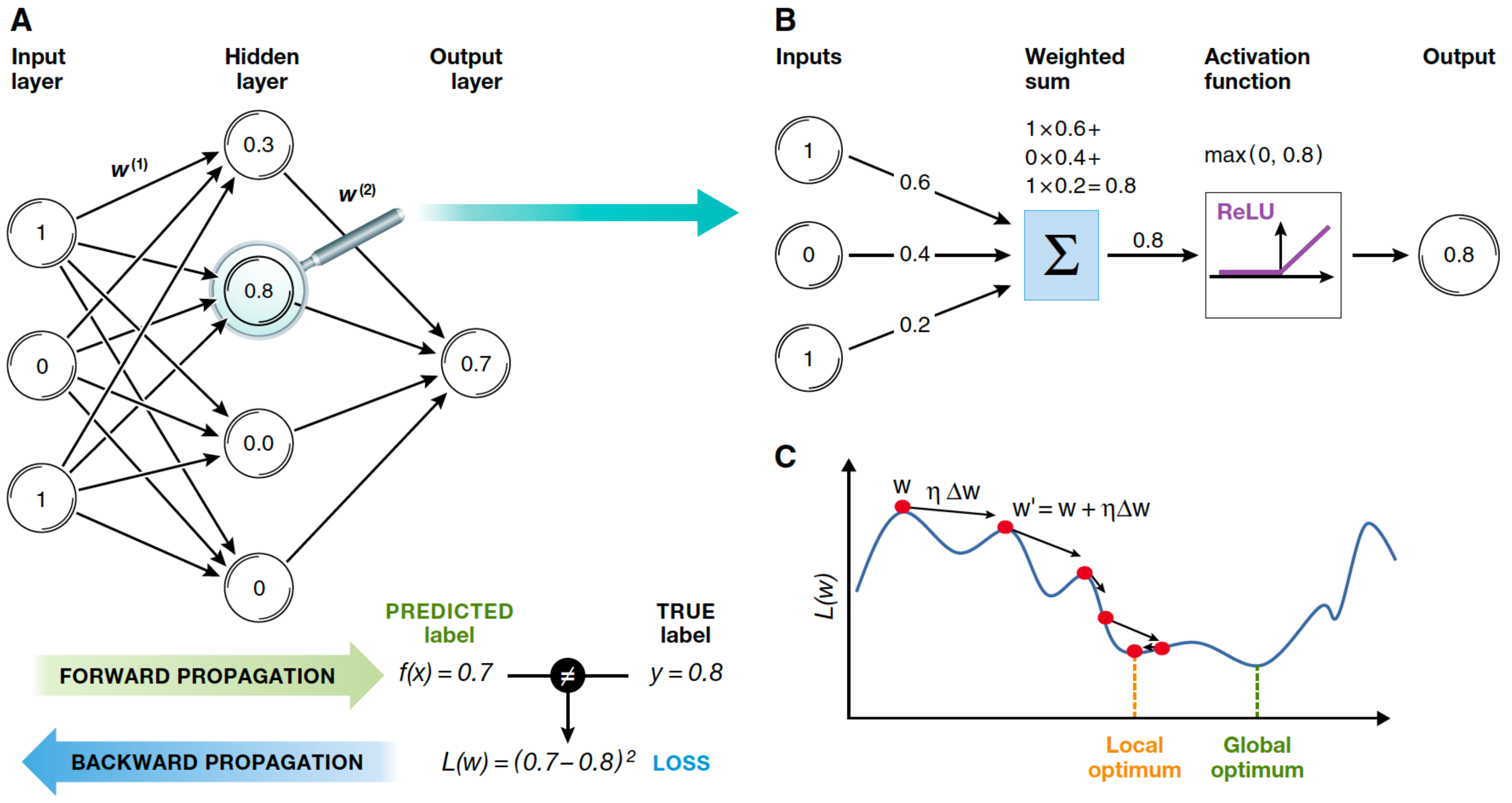
- Single perceptron not useful in practice



- Neural network combines layers of perceptrons
- Learn “hidden” features
- Complex decision boundary
- Tune weights to reduce error
- Train with backpropagation
  - Stanford’s CS231n materials
  - Andrej Karpathy’s gentle introduction
  - CS 760 slides



# Train NN with backpropagation



**Christof Angermueller et al. Mol Syst Biol 2016;12:878**

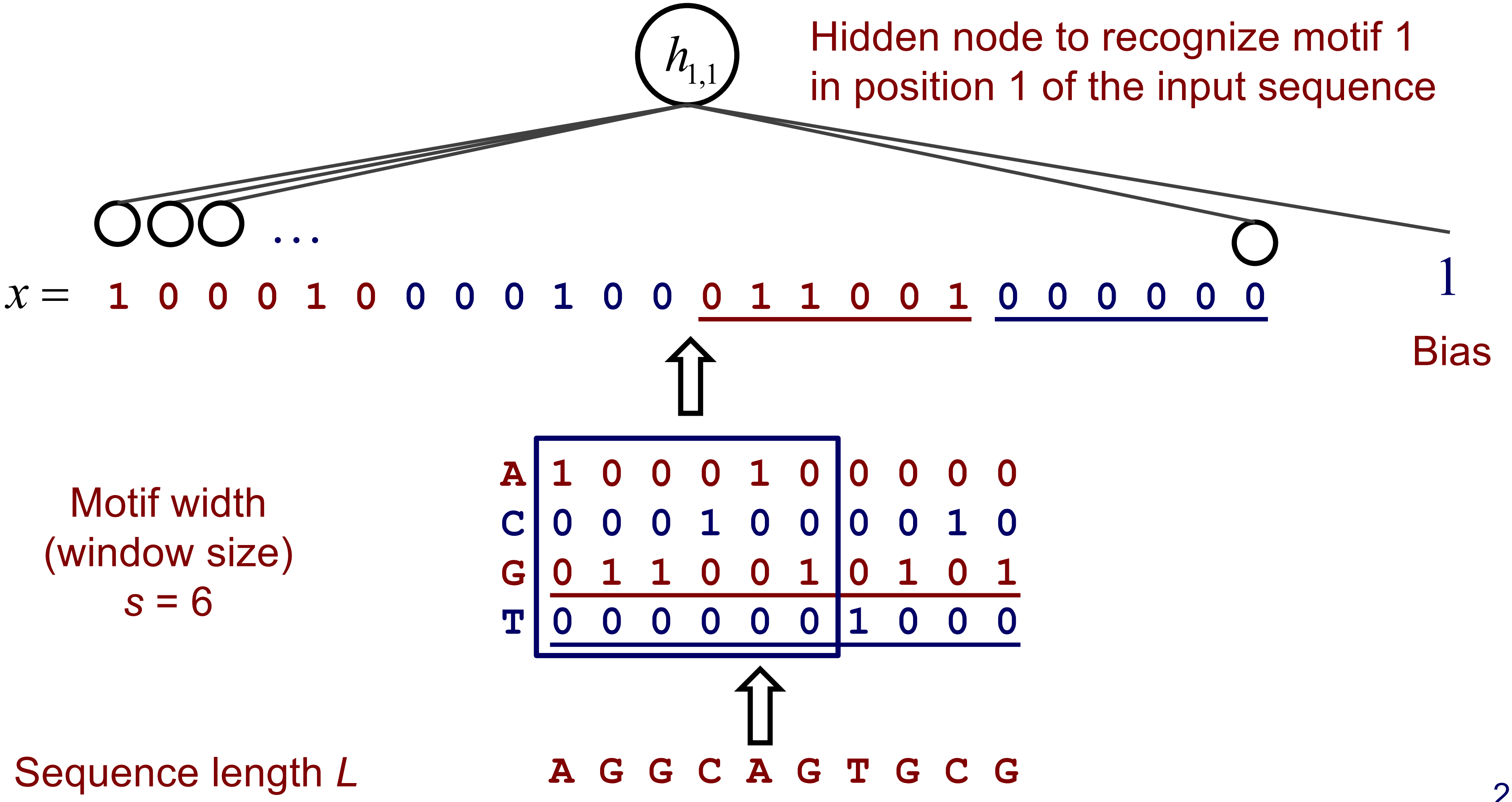
# Neural network examples

- Simple linear decision boundary
- Linear decision boundary fails for XOR
- XOR with one hidden layer
- Complex, non-linear patterns

- Try varying weights, hidden units, and layers
  - What patterns can you learn with 0 hidden layers?
  - 1 hidden layer?
  - More?

# First hidden layer

- First hidden layer scans input sequence
- Activation function fires if “motif” is recognized



Hidden node to recognize motif 1 in position 1 of the input sequence

$x =$ 1 0 0 0 1 0 0 0 0 1 0 0 0 1 1 0 0 1 0 0 0 0 0 0 $1$

Bias

Motif width
(window size)
$s = 6$

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **C** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| **G** | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 |
| **T** | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

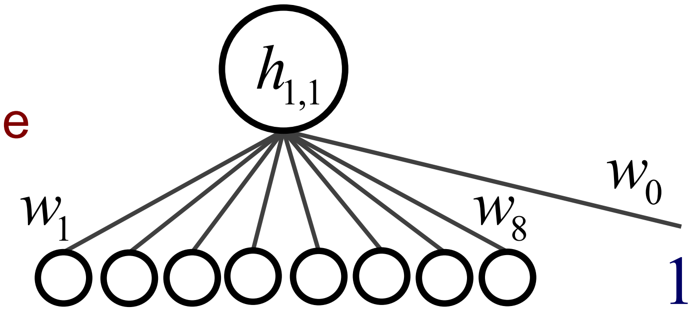
Sequence length $L$

**A G G C A G T G C G**

# First hidden layer example

- Forward pass at the first hidden layer, **input 1**

Hidden node to recognize motif 1 in position 1 of the input sequence



$x^1 = 1\ 0\ 0\ 0\ \underline{0\ 1}\ \underline{0\ 0}$

Bias

For simplicity, *s* = 2

|   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **C** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| **G** | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 |
| **T** | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

**Input sequence 1**

**A G G C A G T G C G**

Current weight vector for $h_{1,1}$

$$w = \begin{bmatrix} w_0 \\ w_1 \\ w_2 \\ w_3 \\ w_4 \\ w_5 \\ w_6 \\ w_7 \\ w_8 \end{bmatrix} = \begin{bmatrix} -1.5 \\ 2 \\ 0.1 \\ 0.4 \\ 0.2 \\ -1.7 \\ 3 \\ -0.5 \\ 0.3 \end{bmatrix}$$

e.g., from PWM of a motif

Weights times input

$$w \cdot x^1 = -1.5 + 2 + 3 = 3.5$$

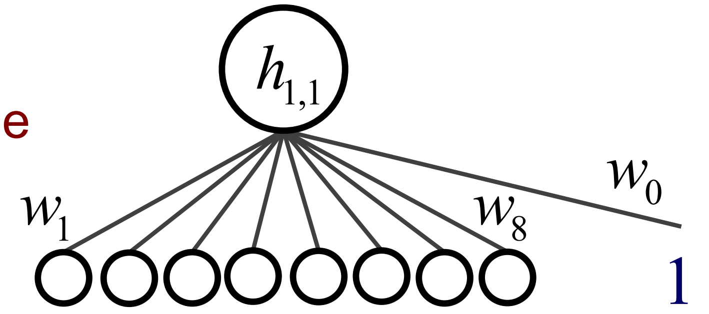
Apply ReLU activation function

$$\max(0, 3.5) = 3.5$$

# First hidden layer example

- Forward pass at the first hidden layer, **input 2**

Hidden node to recognize motif 1 in position 1 of the input sequence



$x^2 = 0\ 0\ 0\ 0\ \underline{1\ 1}\ \underline{0\ 0}$

Bias

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| **C** | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| **G** | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **T** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |

**G G G A C A A C T G**

**Input sequence 2**

Current weight vector for $h_{1,1}$

$$w = \begin{bmatrix} w_0 \\ w_1 \\ w_2 \\ w_3 \\ w_4 \\ w_5 \\ w_6 \\ w_7 \\ w_8 \end{bmatrix} = \begin{bmatrix} -1.5 \\ 2 \\ 0.1 \\ 0.4 \\ 0.2 \\ -1.7 \\ 3 \\ -0.5 \\ 0.3 \end{bmatrix}$$

Weights times input

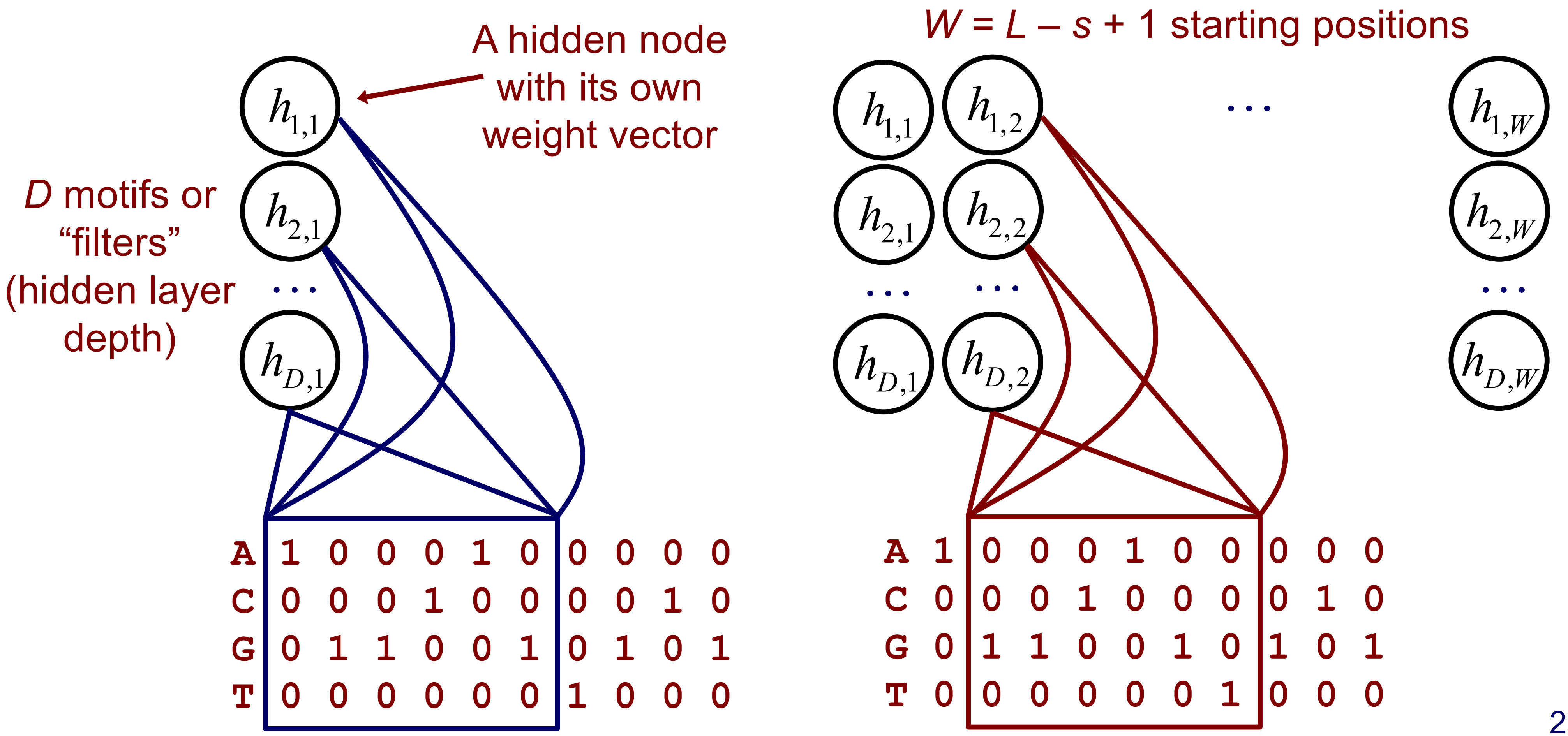
$$w \cdot x^2 = -1.5 - 1.7 + 3 = -0.2$$

Apply ReLU activation function

$$\max(0, -0.2) = 0$$

# First hidden layer

- Multiple hidden nodes to recognize different motifs at a particular position
- Check for motif at each position in sequence



A hidden node with its own weight vector

*D* motifs or "filters" (hidden layer depth)

*W* = *L* – *s* + 1 starting positions

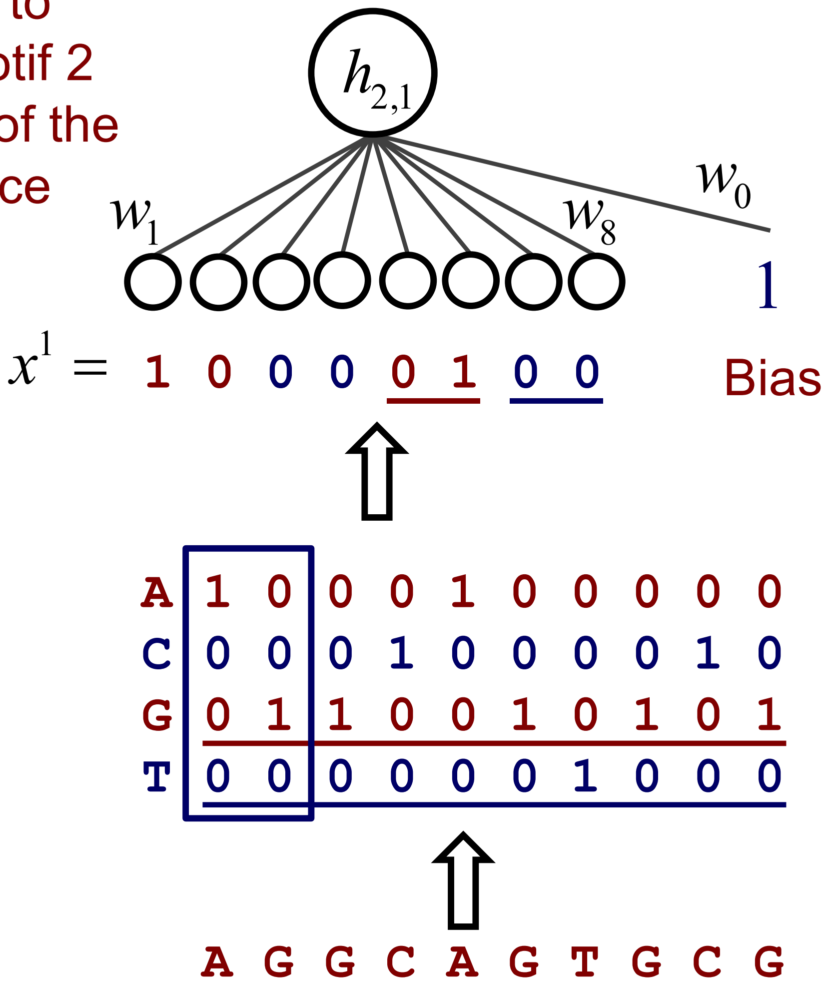
| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **C** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| **G** | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 |
| **T** | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **C** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| **G** | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 |
| **T** | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

# First hidden layer example

- Next filter applied to **input 1**

Hidden node to recognize motif 2 in position 1 of the input sequence



$x^1 = 1\ 0\ 0\ 0\ 0\ 1\ 0\ 0$

|  |  |  |  |  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **C** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| **G** | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 |
| **T** | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

**A G G C A G T G C G**

**Input sequence 1**

Current weight vector for $h_{2,1}$

$$w = \begin{bmatrix} w_0 \\ w_1 \\ w_2 \\ w_3 \\ w_4 \\ w_5 \\ w_6 \\ w_7 \\ w_8 \end{bmatrix} = \begin{bmatrix} 0.5 \\ 0.4 \\ 2.6 \\ 0.1 \\ 0.3 \\ -0.6 \\ 0.3 \\ 5 \\ 3 \end{bmatrix}$$

Weights times input

$$w \cdot x^1 = 0.5 + 0.4 + 0.3 = 1.2$$

Apply ReLU activation function

$$\max(0, 1.2) = 1.2$$

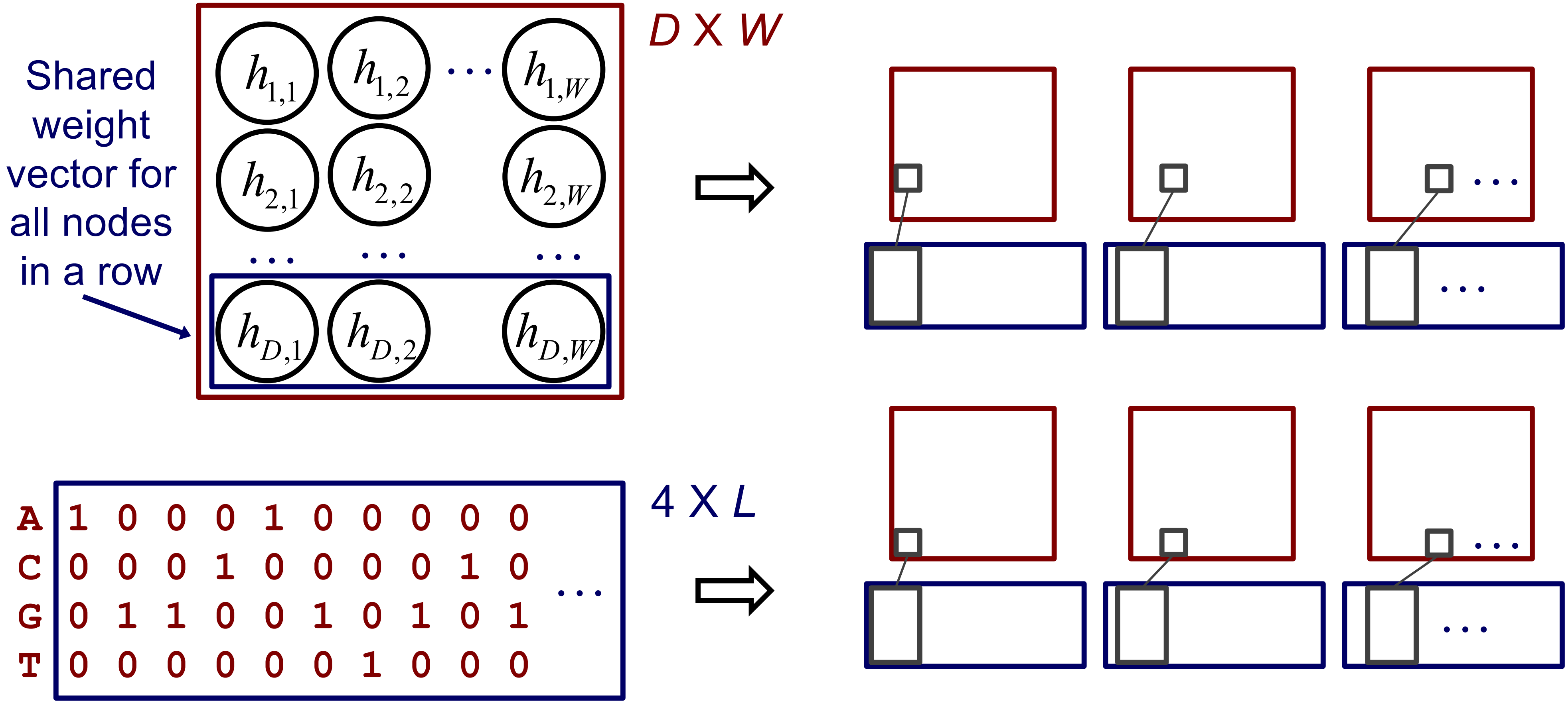
# First layer problems

- We already have a *lot* of parameters
  - Each hidden node has its own weight vector

- We're attempting to learn different motifs (filters) at each starting position
  - *D*W* hidden nodes

# Convolutional layers

- Input sequence and hidden layer as matrices
- Share parameters for all hidden nodes in a row
  - Search for same motif at different starting positions

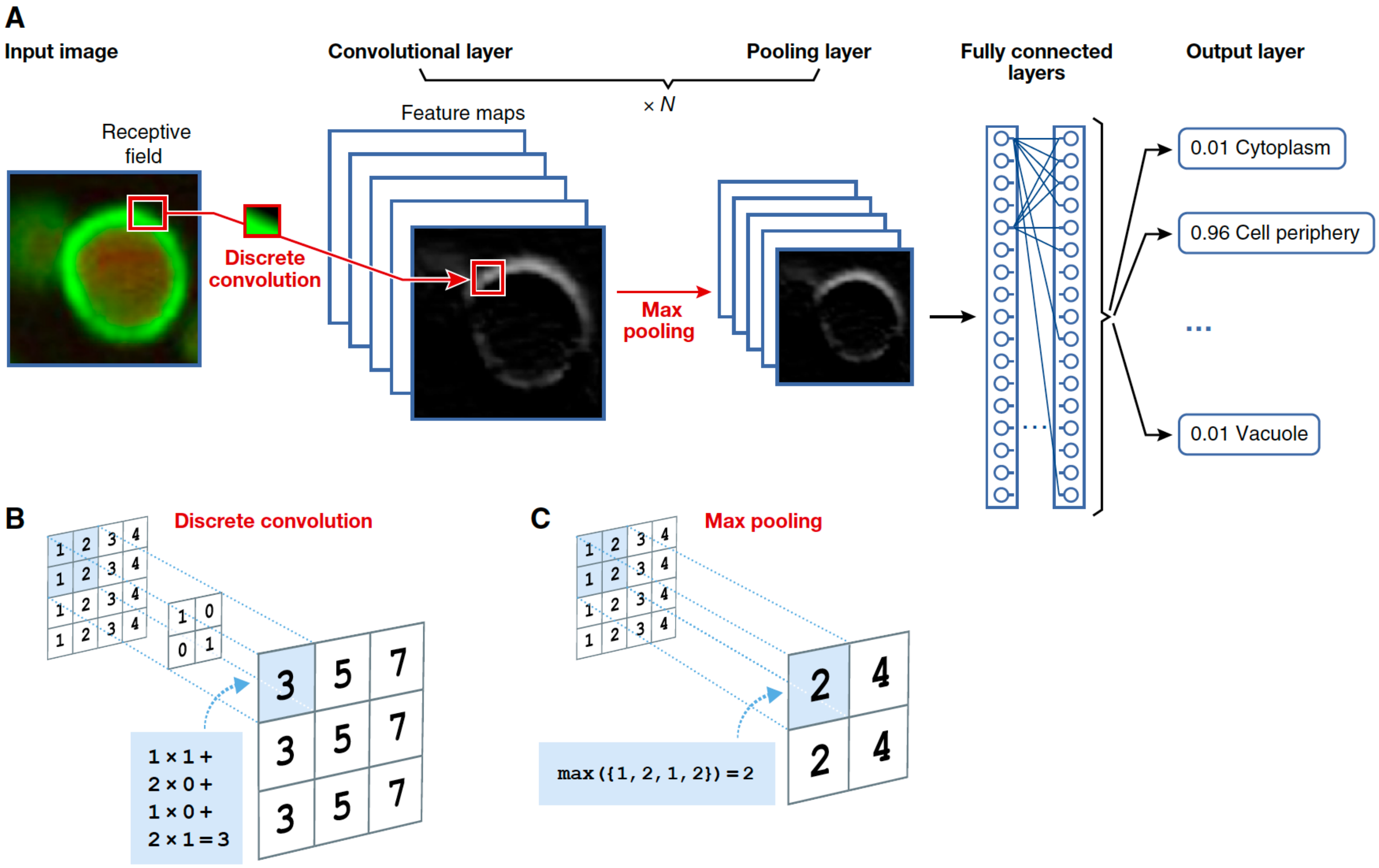


| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **C** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| **G** | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 |
| **T** | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |

# Pooling layers

- Account for sequence context
- Multiple motif matches in a *cis*-regulatory module
- Search for patterns at a higher spatial scale
  - Fire if motif detected anywhere within a window

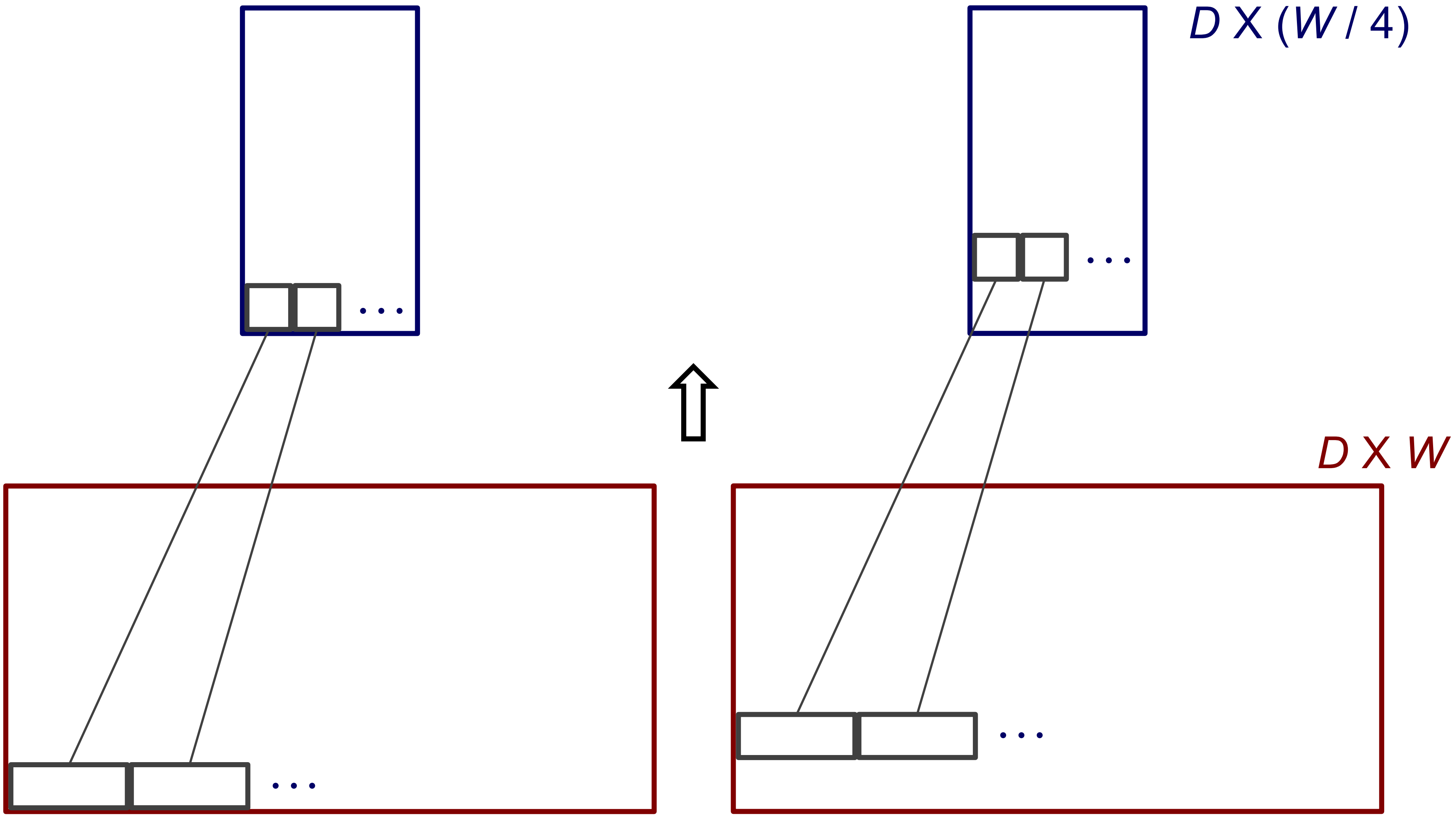
# Convolution and Pooling



Christof Angermueller et al. Mol Syst Biol 2016;12:878

# Pooling layers

- Take max over window of 4 hidden nodes



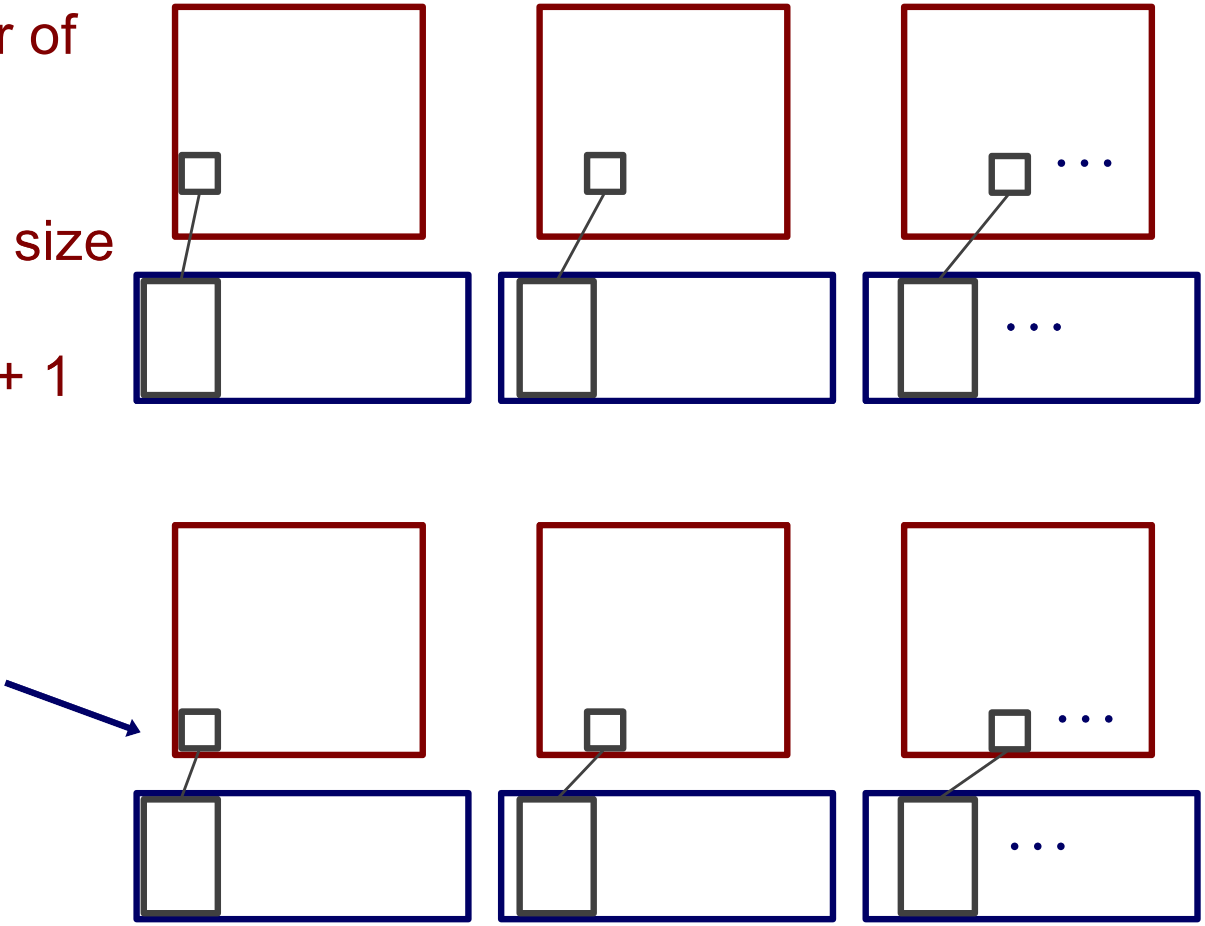
# Subsequent hidden layers

- Next convolutional hidden layer on top of pooling layer

$D'$ is new number of patterns

$s'$ is new window size

$W' = (W / 4) – s' + 1$



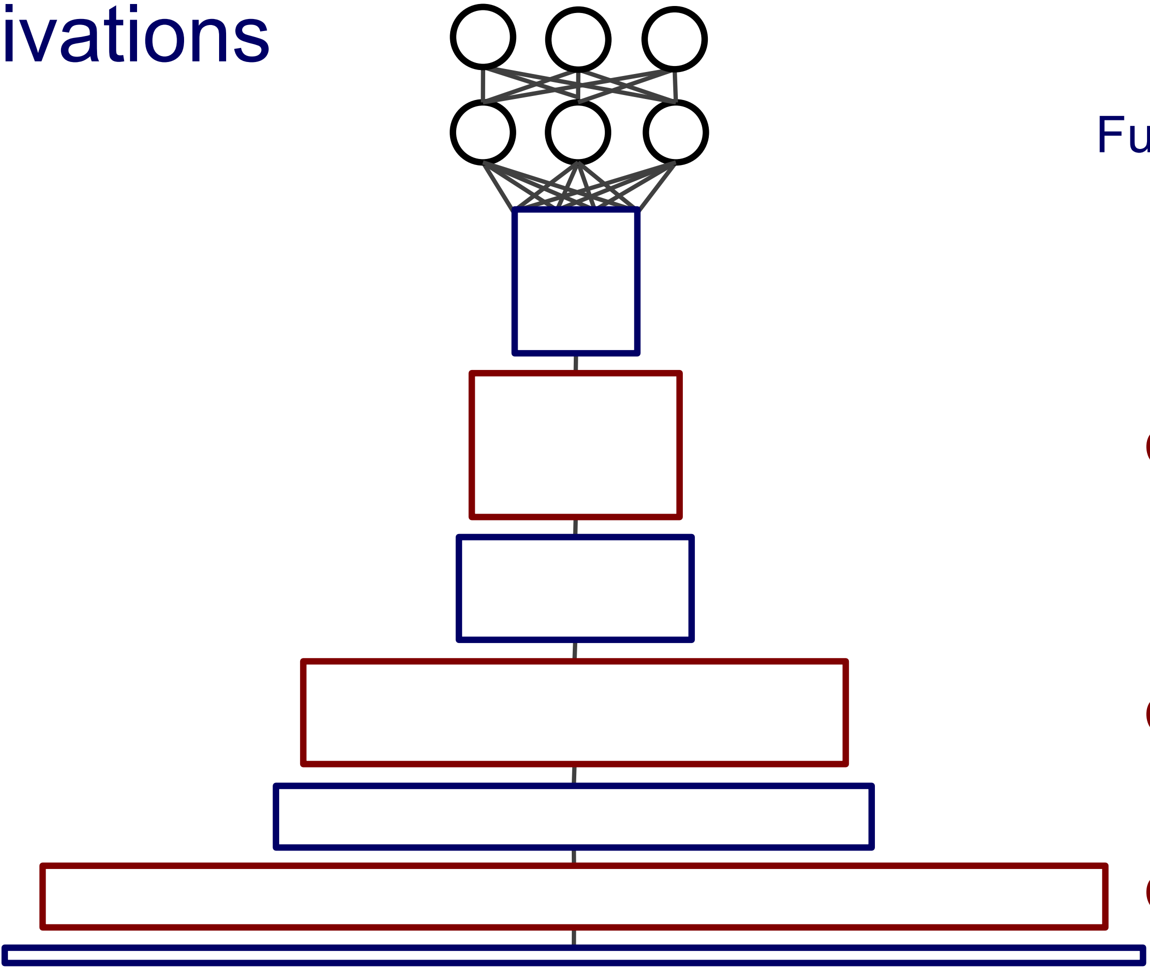
$D'$ X $W'$

$D$ X (W / 4)

Once again, shared weight vector for all nodes in a row

# Full DeepSEA neural network

- Multitask output makes simultaneous prediction for each type of epigenetic data
- ReLU activations



919 classes

Fully connected layer

Pooling layer

Convolutional layer

Pooling layer

Convolutional layer

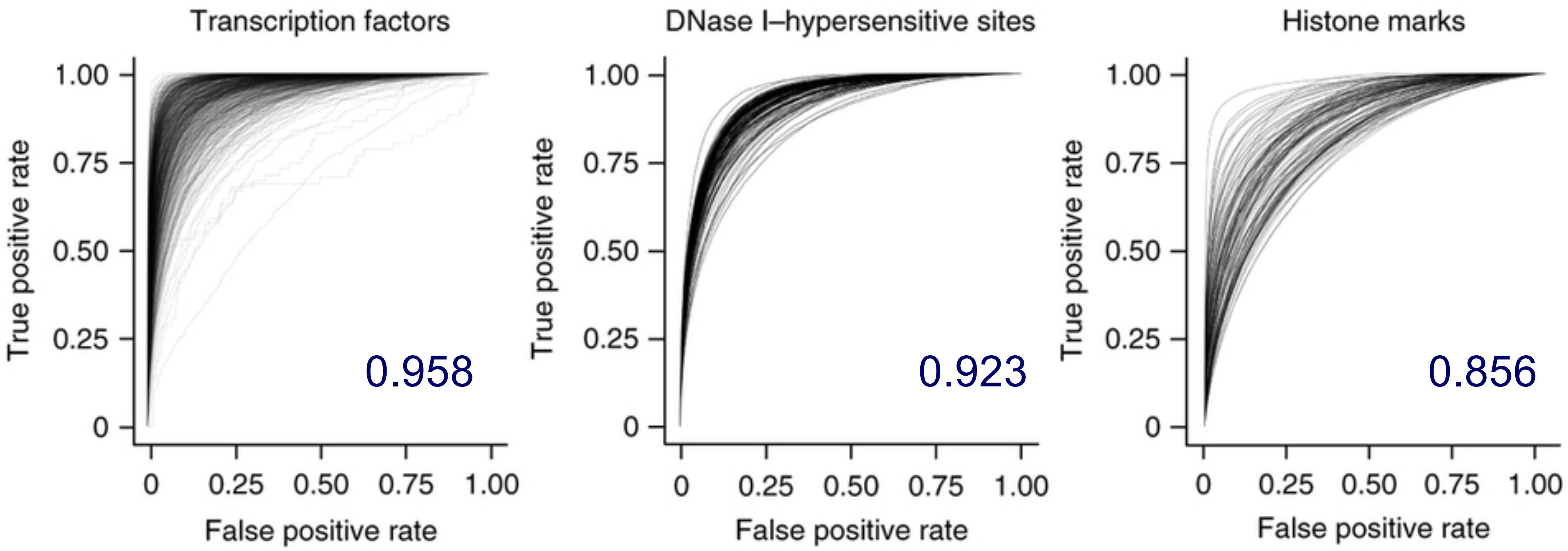
Pooling layer

Convolutional layer

Input sequence

# Predicting epigenetic annotations

- Compute median AUROC for three types of classes



Zhou and Troyanskaya *Nature Methods* 2015

# Predicting functional variants

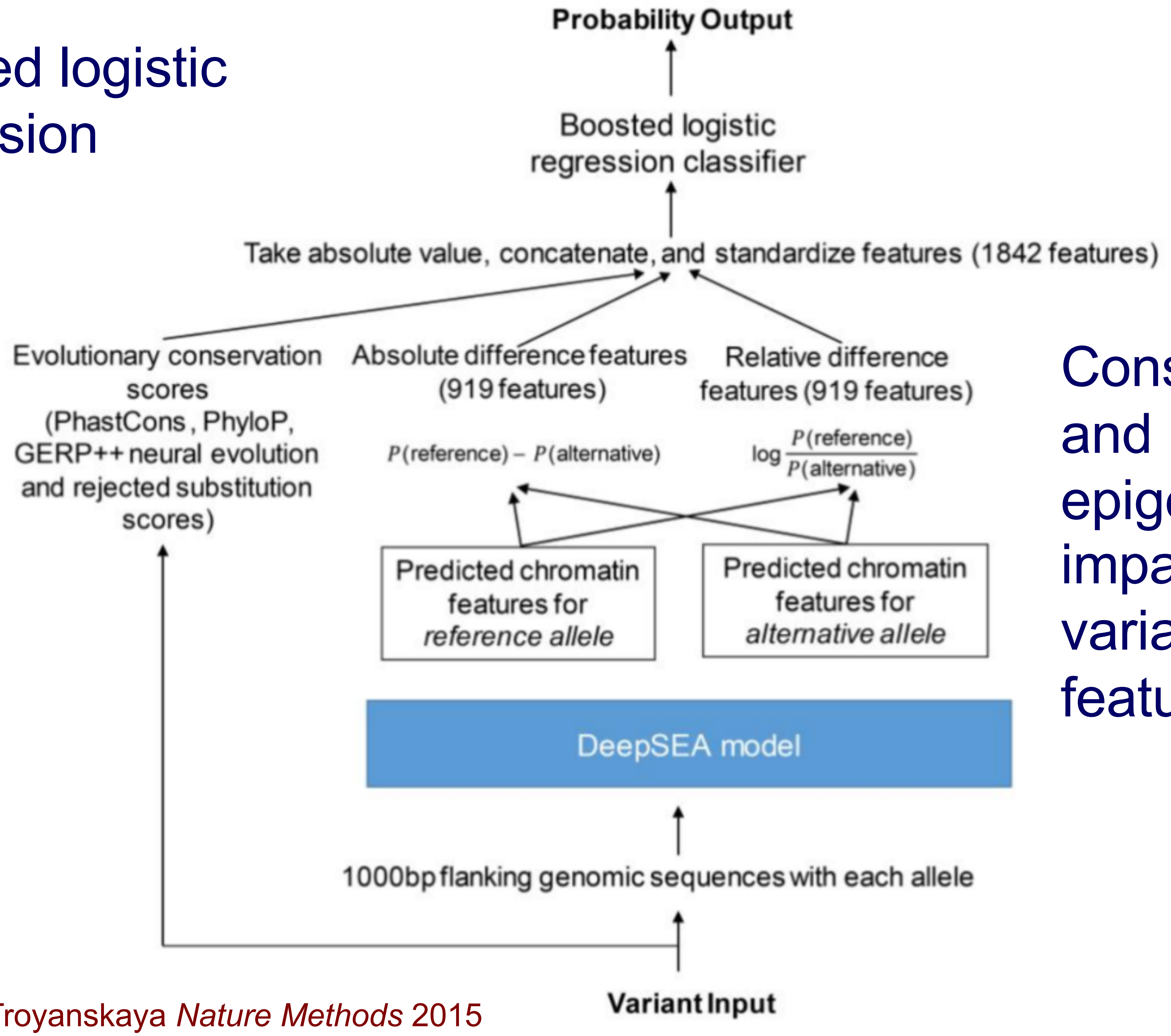
- Can predict epigenetic signal for any novel variant (SNP, insertion, deletion)

- Define novel features to classify variant functionality
  - Difference in probability of signal for reference and alternative allele

- Train on SNPs annotated as regulatory variants in GWAS and eQTL databases

# Predicting functional variants

Boosted logistic regression



**Probability Output**

Boosted logistic regression classifier

Take absolute value, concatenate, and standardize features (1842 features)

Evolutionary conservation scores (PhastCons, PhyloP, GERP++ neural evolution and rejected substitution scores)

Absolute difference features (919 features)

$P(\text{reference}) - P(\text{alternative})$

Relative difference features (919 features)

$\log \frac{P(\text{reference})}{P(\text{alternative})}$

Predicted chromatin features for *reference allele*

Predicted chromatin features for *alternative allele*

DeepSEA model

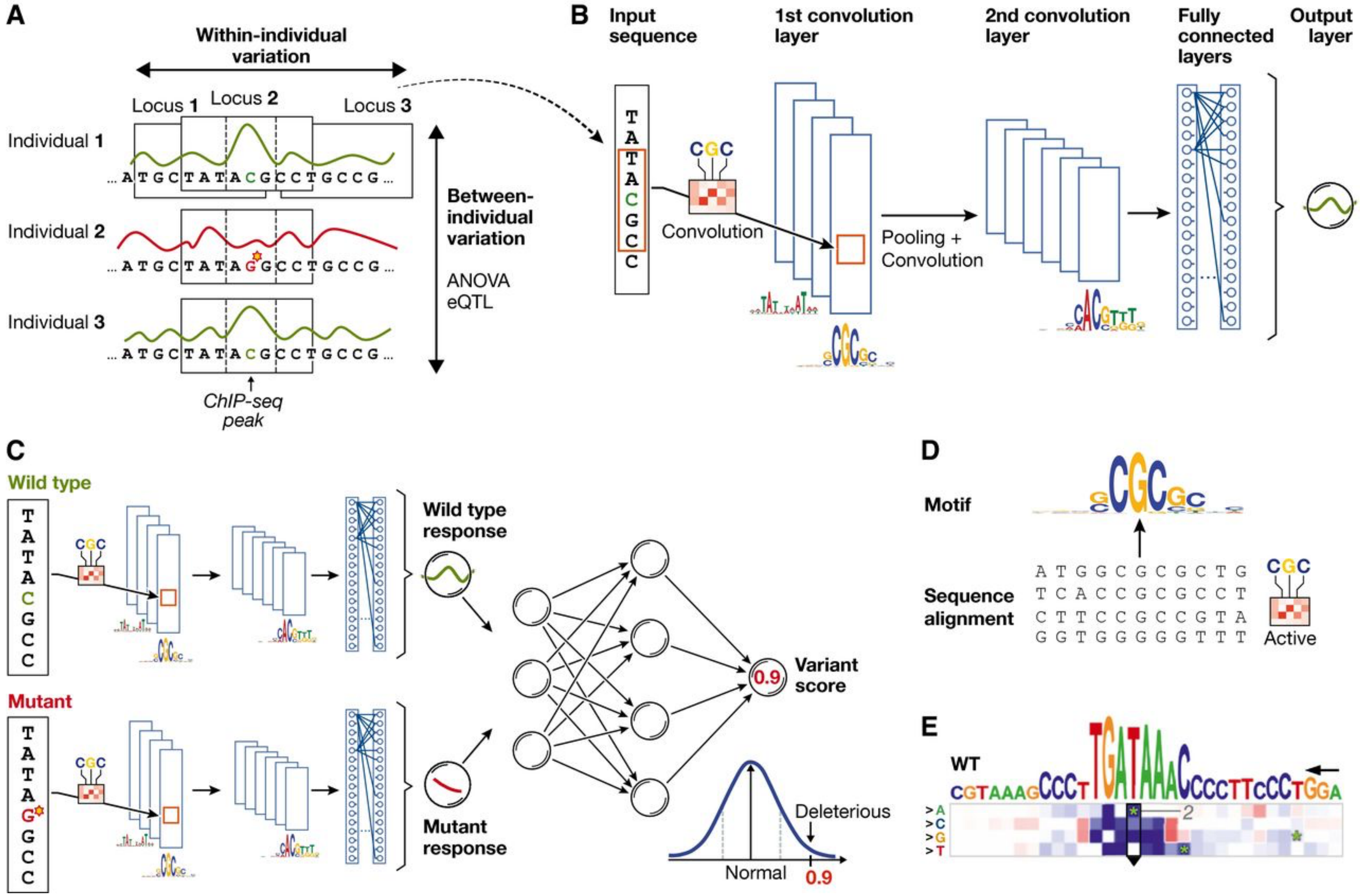
1000bp flanking genomic sequences with each allele

**Variant Input**

Conservation and predicted epigenetic impact of variant as features

Zhou and Troyanskaya *Nature Methods* 2015

# Principles of using neural networks for predicting molecular traits from DNA sequence



**Christof Angermueller et al. Mol Syst Biol 2016;12:878**

# DeepSEA summary

- Ability to predict how unseen variants affect regulatory elements
- Accounts for sequence context of motif
- Parameter sharing with convolutional layers
- Multitask learning to improve hidden layer representations

- Does not extend to new types of cells and tissues
- AUROC is misleading for evaluating genome-wide epigenetic predictions

# Predicting new TF-cell type pairs

Training data

| TF | Cell type |
|---|---|
| A | 1 |
| A | 2 |
| A | 3 |
| B | 3 |
| B | 4 |
| C | 1 |
| C | 4 |

- DeepSEA cannot predict pairs not present in training data
  - Can predict TF A in cell type 1
  - Not TF A in cell type 4

- New methods can
  - TFImpute
  - FactorNet
  - Virtual ChIP-seq

# Deep learning is rampant in biology and medicine

- Network interpretation: DeepLIFT
- Protein structure prediction
- Cell lineage prediction
- Variant calling

- Model zoo: Kipoi

- Comprehensive review: deep-review