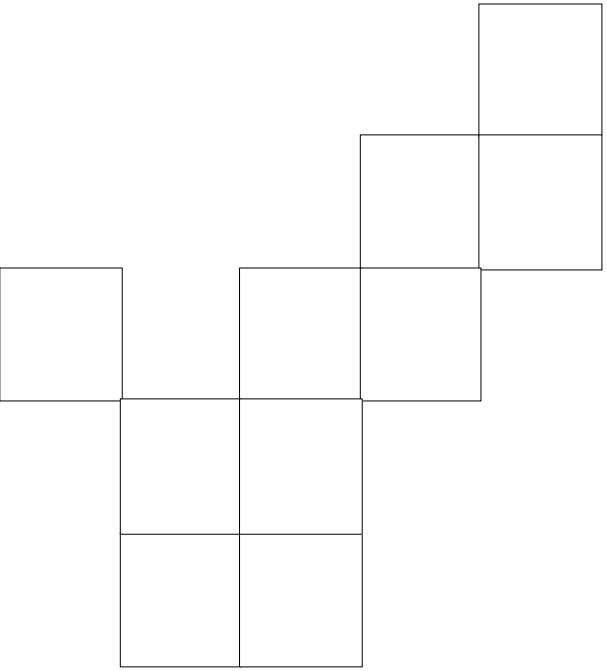

Prediction of noncoding RNAs with RNAz

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April 4, 2007

What is non-coding RNA (ncRNA)?

- RNA molecules that are not translated into proteins
- Size range from 20 to1000's of nucleotides in length
- Significantly gained scientific interest since 1990's
  - Originally thought as intermediates or accessories in protein biosynthesis
    - Little was known of their importance
    - Majority of research and funding towards protein coding RNA (messenger RNA)
  - Improved scientific methods and sequencing techniques
    - Led to the discovery of novel functions
    - Led to further classifications of RNA
  - Discovery of ten of thousands of ncRNA expressed in human cells
    - more ncRNA's expressed in human cells than protein coding RNA's.

Function of ncRNA?

- Structural, regulatory and catalytic molecules of protein biosynthesis
- Maturation of mRNA, tRNA and rRNA
- X-chromosome inactivation in mammals
- Gene regulation

Types of ncRNA

- Transfer RNA (tRNA)
  - ~73 – 93 nucleotides in length
  - Function
    - Transfer specific amino acid to ribosomal site during protein synthesis (translation)
  - Specialized L-shape structure
    - Allows tRNA to “dock” onto ribosomal site for amino acid transfer

Types of ncRNA (cont.)

- Ribosomal RNA (rRNA)
  - Primary constituent of ribosomes
    - Ribosomes primary role is to assemble polypeptides from amino acids (translation)
    - Ribosomal proteins combined with rRNA to create ribosome
  - Make up the majority of RNA found within a typical cell
- Small nuclear RNA (snRNA)
  - Located in nucleus of eukaryotic cells
  - Function
    - RNA splicing
    - Regulation of transcription factors
    - Maintaining telomeres

Types of ncRNA (cont.)

- Small Nucleolar RNA (snoRNA)
  - Located in the nucleolus
    - Ribosomes primary role is to assemble polypeptides from amino acids (translation)
    - Ribosomal proteins combined with rRNA to create ribosome
  - Function
    - Enhance functionality of mature RNA
      - chemical modifications to rRNA and other RNA genes (ex. methylation)
- Micro RNA
  - ~20 – 23 nucleotides in length
  - Single stranded
    - Complimentary to one or more messenger RNA (mRNA)
  - Function
    - Regulates gene expression
      - anneals itself to mRNA inhibiting translation

Why is it hard to predict non-coding RNA?

- Unlike protein coding genes, functional RNAs lack statistical signals for reliable detection from primary sequences
- There is no protein product for which the ncRNAs are coding
  - No evolutionary constraints on protein product
  - Constraints come in secondary RNA structure
    - Can be conserved even with substantial changes to primary DNA sequence

How do ncRNA prediction programs overcome this problem?

- QRNA – uses pairwise alignment, but low reliability
- MSARI – uses multiple sequence alignments of 10-15 sequences with high sequence diversity; highly accurate
- RNAz – combines sequence alignment of 2-4 sequences with measures of:
  - Structural conservation
  - Thermodynamic stability

RNAz

- Predicts noncoding RNA sequences
- Relies on two features of structural noncoding RNAs:
  - Thermodynamic stability
  - Secondary structure conservation
- Uses comparative sequence analysis of 2-4 sequences
- Builds on other RNA programs to accomplish goal:
  - RNAFOLD – folding single sequences
  - RNAALIFOLD – consensus folding of aligned sequences
  - LIBSVM – support vector machine (SVM) learning

Thermodynamic stability

- Measure mean free energy (MFE)
- Compares mean free energy of given sequence to random sequences of same length and base composition
- Z-score calculated as:

  $z = (m - \mu)/\sigma$

  where μ and σ are the mean and standard deviations of the random sequences, respectively.

- Negative z scores indicate that a sequence is more stable than expected by chance.

Structural conservation

- Uses RNAalifold
  - Like RNAfold except augmented with covariance information
- For covariance information, compensatory mutations (e.g. a CG pair mutates to a UA pair) and consistent mutations (e.g. AU mutates to GU) give a bonus of energy while inconsistent mutations (e.g. CG mutates to CA) yield a penalty of energy
- Results in consensus MFE E_A.
- RNAz compares E_A to average MFE of individual sequences (E_{avg})
- Structural conservation index calculated as:

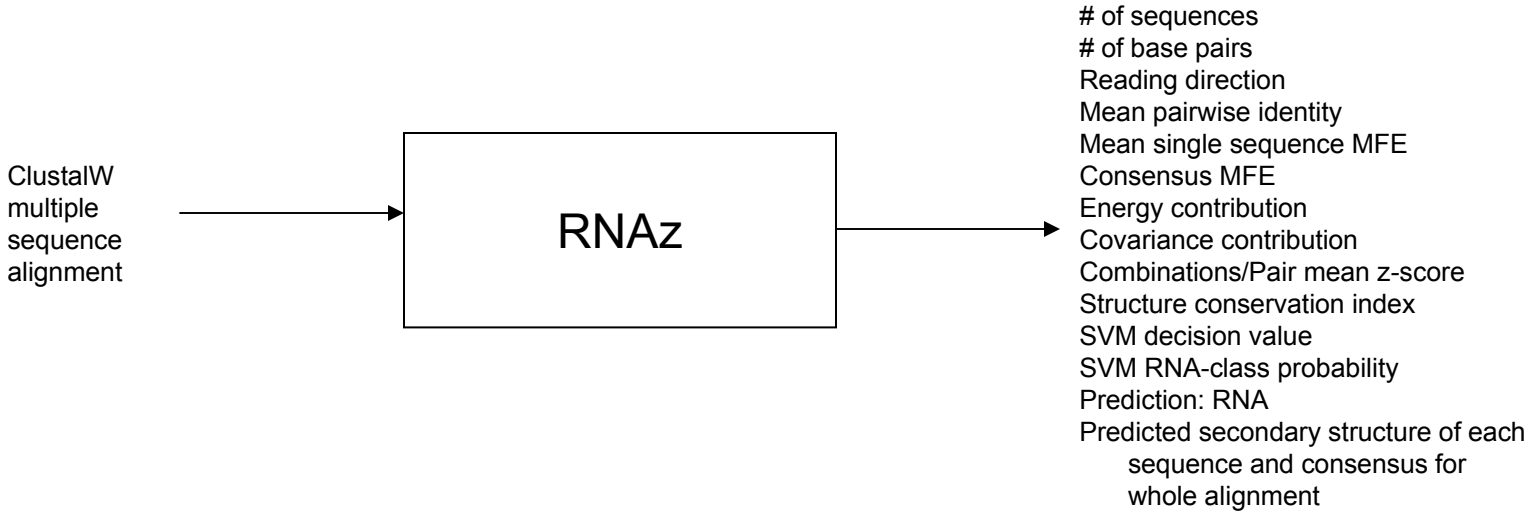
$$SCI = E_A / E_{avg}$$

- SCI high => sequences fold together equally well as fold individually
- SCI low => no consensus fold

Combining z and SCI scores

- Z- and SCI scores used to classify the alignment as “structural noncoding RNA” or “other” using Support Vector Machine (SVM) learning algorithm
- Trained using a large set of well-known noncoding RNA sequences

RNAz: Input and Output

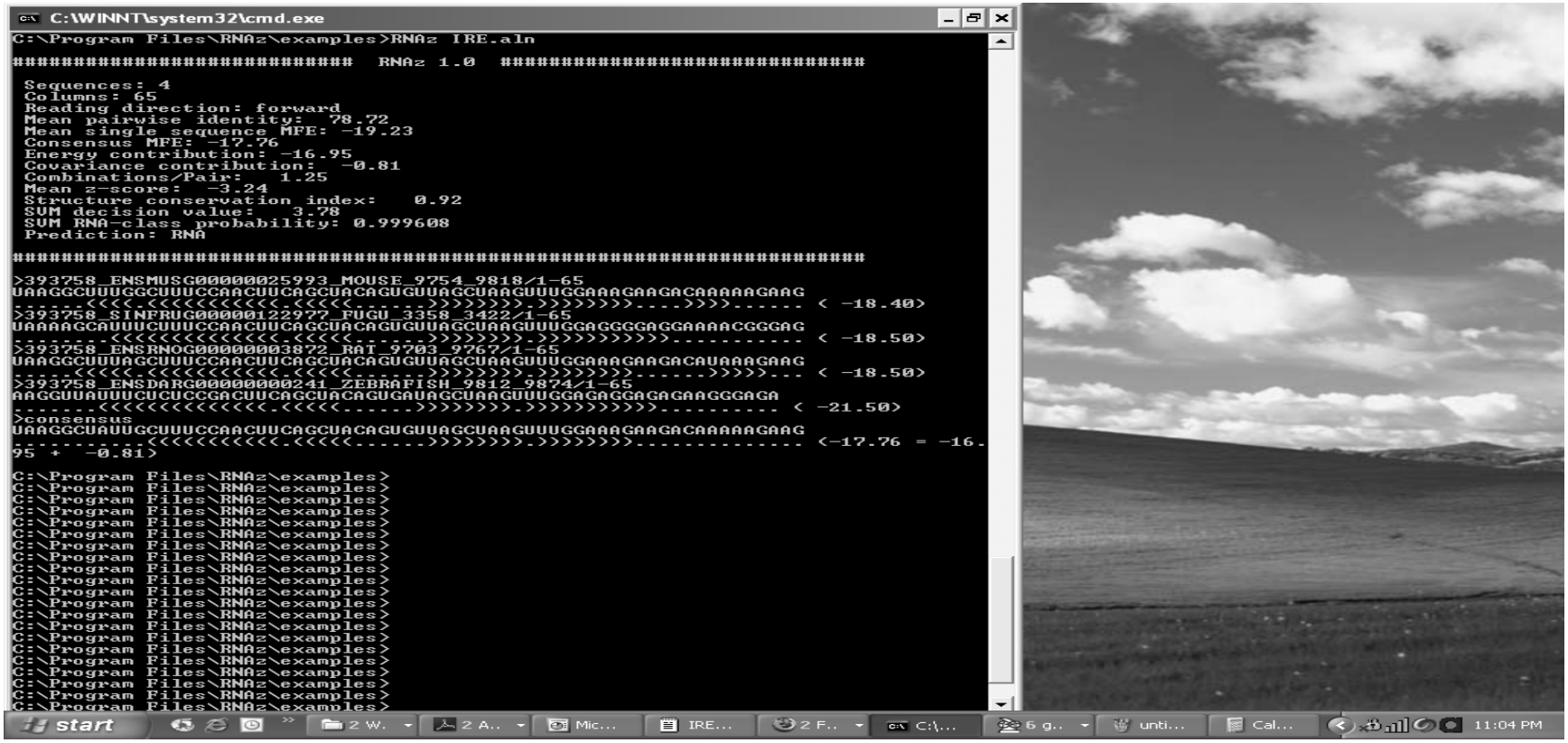


- Input requires aligned sequences in ClustalW or MAF formats
- Output provides:
  - Properties of sequences (number of sequences and base pairs, reading direction, pairwise identity)
  - Thermodynamic scores (MFE for sequences and consensus, energy contribution, covariance contribution, z-scores)
  - Secondary structure conservation (structure conservation index)
  - Classification prediction (SVM decision value, class probability, prediction)
  - Predicted secondary structure of each sequence and consensus

Example: Iron Response Element (IRE) RNA Input

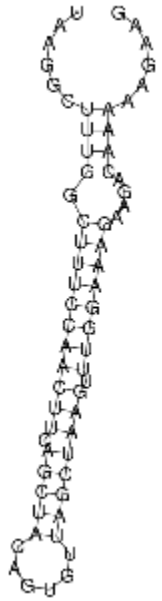
```
CLUSTAL W (1.83) multiple sequence alignment


sacCer1
   GCCTTGTTGGCGCAATCGGTAGCGCGTATGACTCTTAATCATAAGGTTAGGGGTTCGAGC
sacBay
   GCCTTGTTGGCGCAATCGGTAGCGCGTATGACTCTTAATCATAAGGTTAGGGGTTCGAGC
sacKlu
   GCCTTGTTGGCGCAATCGGTAGCGCGTATGACTCTTAATCATAAGGCTAGGGGTTCGAGC
sacCas
   GCTTCAGTAGCTCAGTCGGAAGAGCGTCAGTCTCATAATCTGAAGGTCGAGAGTTCGAAC
   ** *   * ** ** **** ** ****  * *** *****  ****    * ****** *


sacCer1         CCCCTACAGGGCT
sacBay          CCCCTACAGGGCT
sacKlu          CCCCTACAGGGCT
sacCas          CTCCCCTGGAGCA
                * **    * **
```

Example: Iron Response Element (IRE) RNA Output



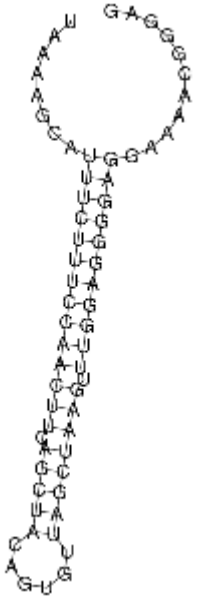
IRE RNA Structures Using RNA Fold

Mouse



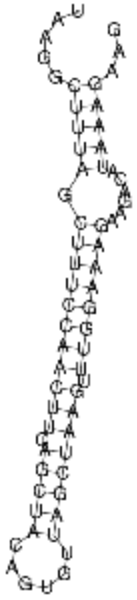
RNAFOLD: MFE = -19.66 kcal/mol

Fugu



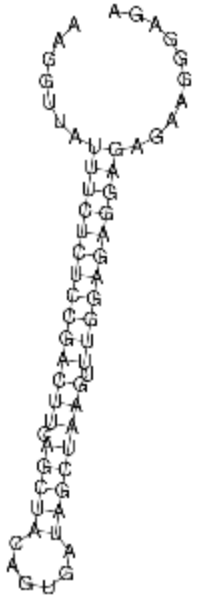
MFE = -19.70 kcal/mol

Rat



MFE = -19.44 kcal/mol

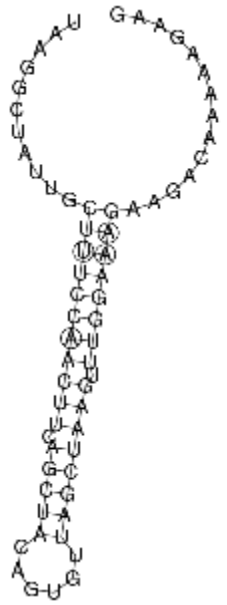
Zebrafish



MFE = - 22.94 kcal/mol

Average MFE = -20.43 (vs. -19.23 for output of RNAz)

Consensus Folding via RNAALIFOLD

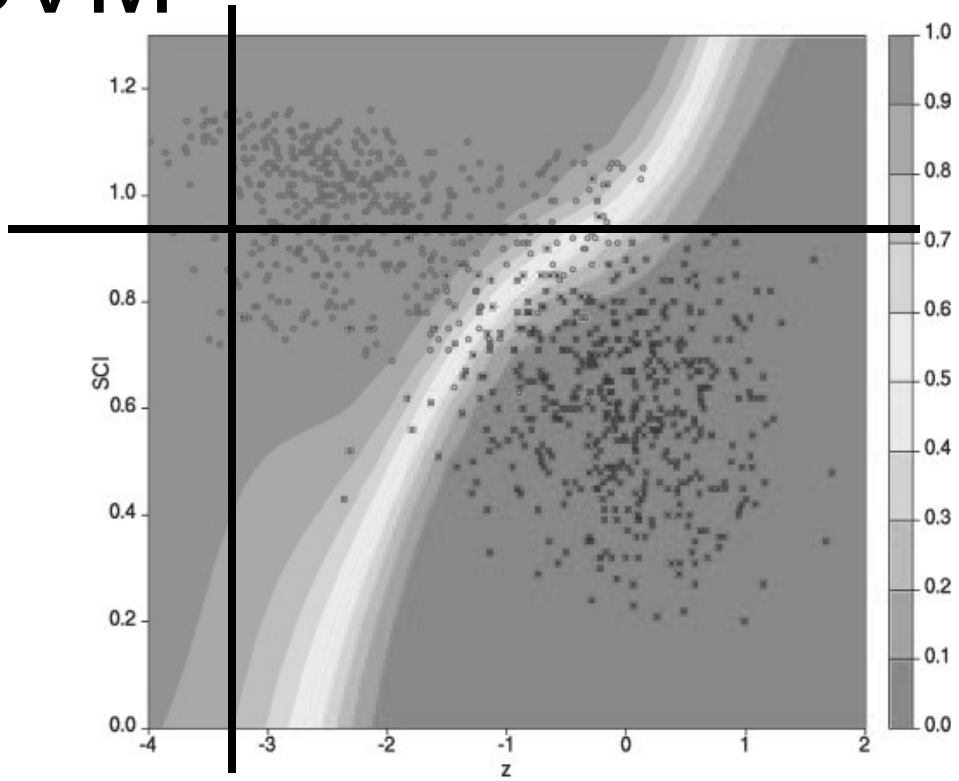


MFE = E_A = -17.76 kcal/mol

SCI = E_A / E_{avg} = -17.76/(-19.23) = 0.92

Fold together equally well as individually

Classification of Z scores and SCI using SVM



Green = high probability of structural ncRNA

Red = low probability of structural ncRNA

- Z score = -3.24
- SCI = 0.92

High probability of structural noncoding RNA

3 Algorithms in RNAz

- Calculation of z-score
- Calculation of SCI
- SVM for classification of consensus as “structural noncoding RNA” or “other”

We will explain each of these algorithms in turn

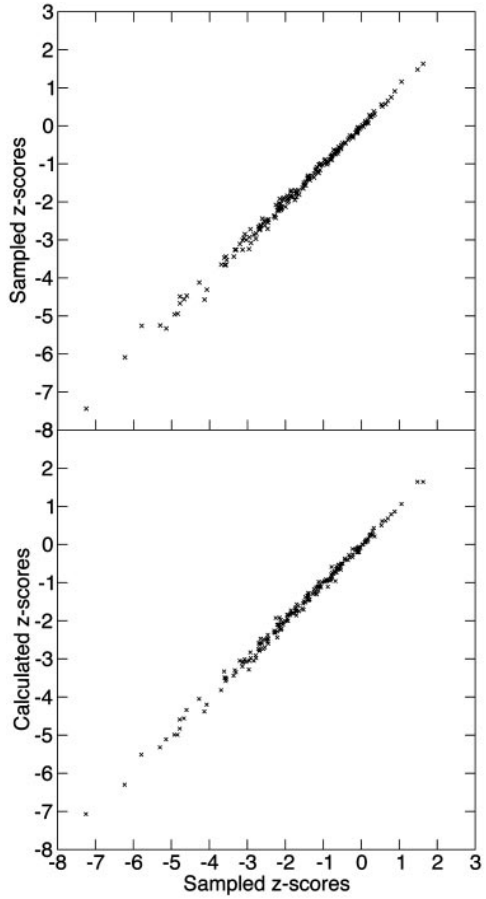
Calculation of z-score

- Generated synthetic combinations of different length and base composition
  - 50 – 400 nucleotides in steps of 50 (8 sizes)
  - GC/AT, A/T, G/C ratios of sequences ranging from 0.25 to 0.75 in steps of 0.05 (11 percentages per ratio type)
  - 10,648 combinations (= 8 x 11 x 11 x 11)
- For each combination, generate 1000 random sequences and calculated mean and standard deviation of MFE
- Used SVM library LIBSVM to train 2 regression models for mean and standard deviation (μ and σ) rather than using random sampling. Verified accuracy by comparison of SVM algorithm and sampling.
- Z score calculation:

$$z = (MFE - \mu)/ \sigma$$

where μ is the mean of sequences with a given length and base composition and sigma is the standard deviation

Accuracy of using SVM for Z-score Calculation



- Comparison of z scores through two methods:
  - Sampling
    - 100 sequences from random locations in human genome
    - 100 known ncRNAs from Rfam database
  - Using SVM regression model
- SVM model eliminates need for extensive sampling

Calculation of SCI

- SCI calculation:

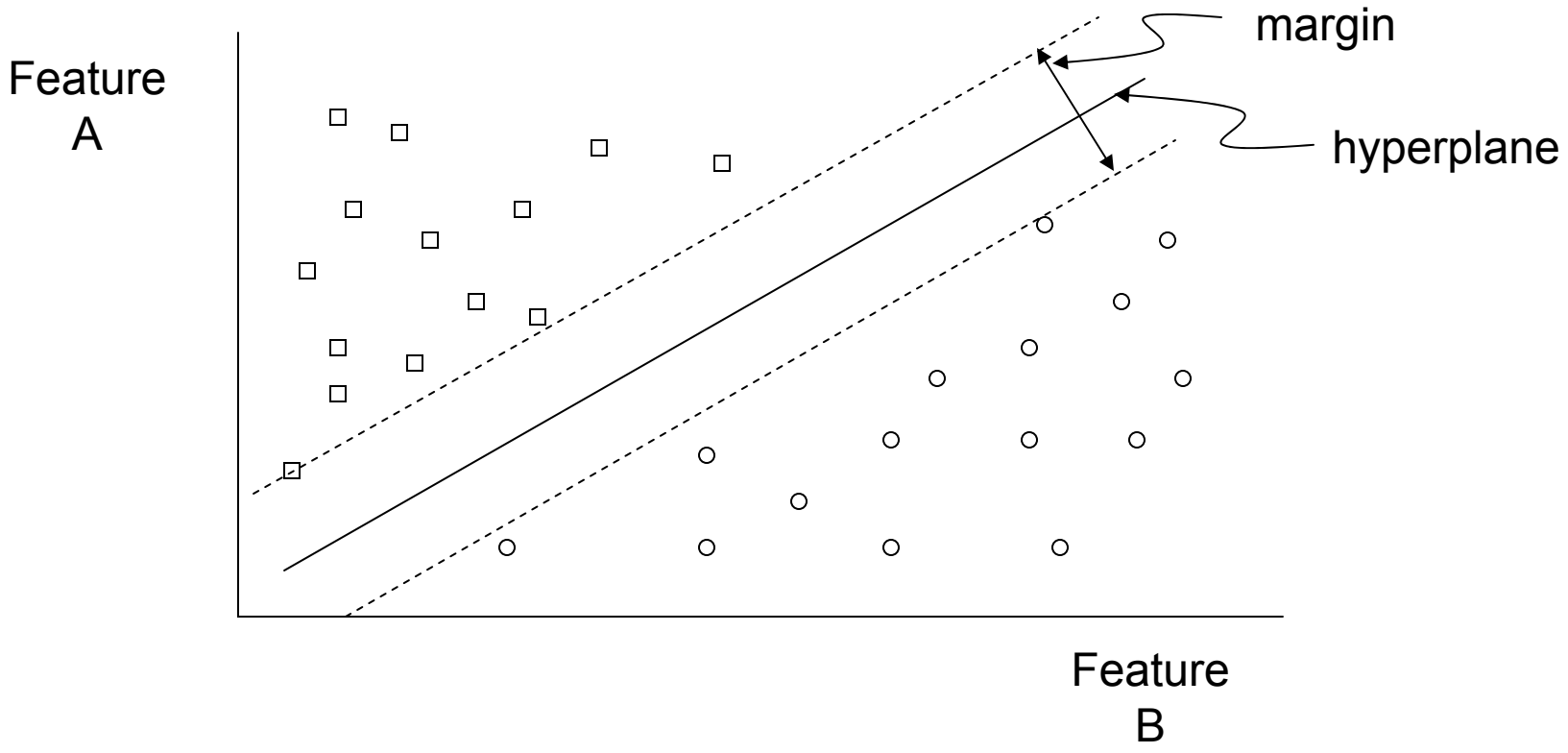
$$SCI = E_A / E_{avg}$$

where E_A is the consensus MFE of the aligned sequences and E_{avg} is the average MFE of the individual sequences

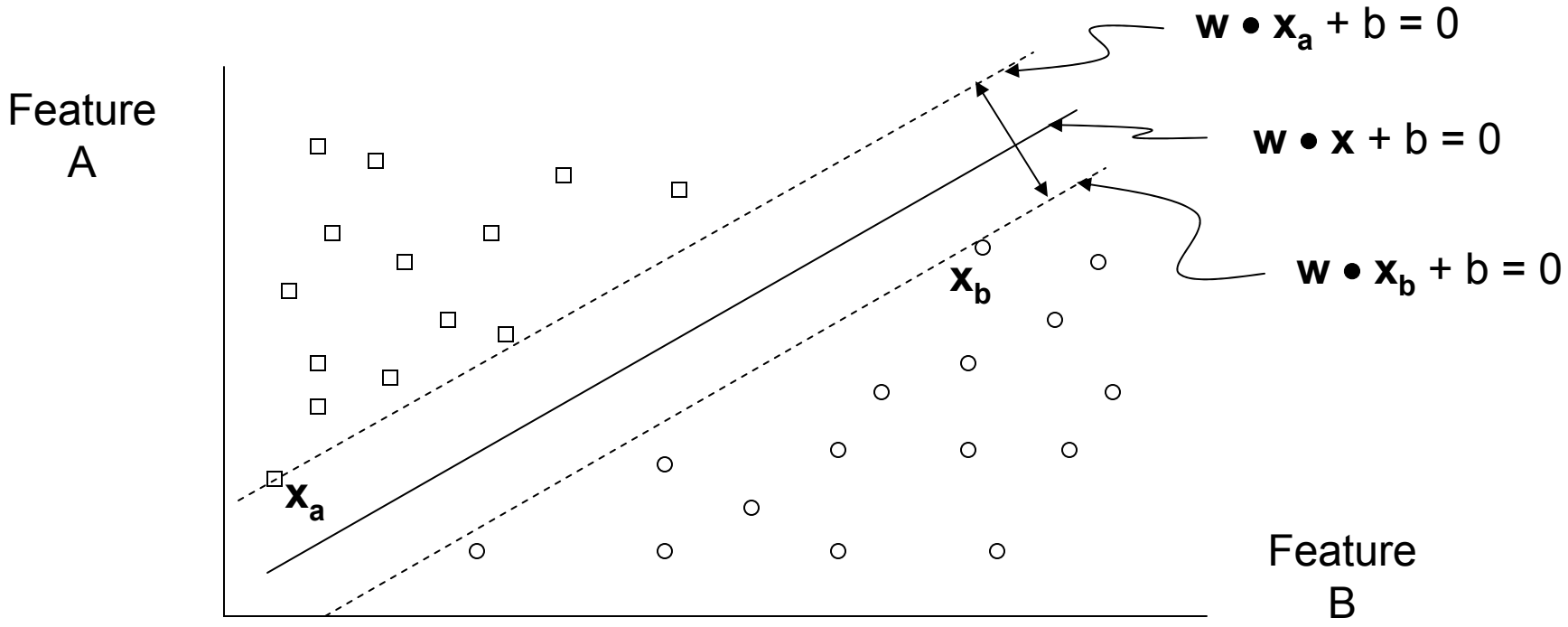
- E_A calculated through RNAALIFOLD

Support Vector Machines

- Support Vector Machines provide a means of classifying data into different classes or categories
- Binary classifier separates data into two separate classes
- Goal: Find hyperplane with the maximum margin that separates two classes of data
  - Reduces impact of changes in underlying model
  - Minimizes false positives



Binary Linear SVM



Each value represented by tuple $(\mathbf{x}_i, y_i)$ (I = 1, 2 in this example) where $\mathbf{x}_i = (x_{i1}, x_{i2}, \ldots, x_{id})^T$ corresponds to the attribute set for the ith value. y_i can either be 1 or -1 to denote the binary choice.

Decision boundary of linear classifier has form:

$$\mathbf{w} \bullet \mathbf{x} + b = 0$$

where **w** and b are parameters in the model.

For test value **z**:

$$y = \begin{cases} 1, \text{ if } \mathbf{w} \bullet \mathbf{z} + b \geq 0 \\ -1, \text{ if } \mathbf{w} \bullet \mathbf{z} + b < 0 \end{cases}$$

Training with SVM

Train model with data that has already been classified

- For this presentation, this means known ncRNA and know non-ncRNA.
- For a linear model, the training data is used to set **w** and b (after scaling) such that:

$$\min f(\mathbf{w}) = ||\mathbf{w}||^2 / 2 \text{ subject to } y_i(\mathbf{w} \bullet \mathbf{z}_i + b) \geq 1,\ I = 1, 2, \ldots, N$$

  - $\mathbf{w} \bullet \mathbf{z} + b \geq 1$ if $y_i = 1$ (i.e., for known ncRNA),
  - $\mathbf{w} \bullet \mathbf{z} + b < 1$ if $y_i = -1$ (i.e., for known non-ncRNA)
  - Must also maximize the margin:
    - Equivalent to:

$$\min_{\mathbf{w}} f(\mathbf{w}) = ||\mathbf{w}||^2 / 2 \text{ subject to } y_i(\mathbf{w} \bullet \mathbf{z}_i + b) \geq 1,\ I = 1, 2, \ldots, N$$

Two Additional SVM Issues

- Two additional SVM issues need explanation for this paper:
  (1) What if training data not outside of margin because of noise in the training data?
  (2) What if two classes cannot be separated by a line?

- To handle the first issue, positive slack variables are added into the constraints of the f(w) optimization such that:

$$\min_{\mathbf{w}} f(\mathbf{w}) = ||\mathbf{w}||^2 / 2 + C\left(\sum_{i=1}^{N} \xi_i\right)^k \text{ subject to } y_i(\mathbf{w} \bullet \mathbf{z}_i + b) \geq 1 - \xi_i,\ I = 1, 2, \ldots, N$$

  where C and k represent penaties for misclassifying training instances.

- To handle the second issue, we transform the data from its original space to a transformed space with a mapping function $\Phi(\mathbf{x})$ where there is a linear hyperplane between the two datasets. This mapping has the property:

$$K(\mathbf{u},\mathbf{v}) = \Phi(\mathbf{u}) \bullet \Phi(\mathbf{v}) = (u \bullet v + 1)^2$$

  where K is a **kernel function**.

- Only certain kernel functions can be used. Some common ones include:

  - Polynomial: $K(\mathbf{x},\mathbf{x}) = (\gamma \mathbf{x}^T\mathbf{x} + r)d,\ \gamma > 0$,
  - Radial basis function: $K(\mathbf{x},\mathbf{x}) = \exp(-\gamma\ ||\ \mathbf{x} - \mathbf{x}||^2),\ \gamma > 0$,
  - Sigmoid $K(x,x) = \tanh(\gamma\ \mathbf{x}^T\mathbf{x} + r)$

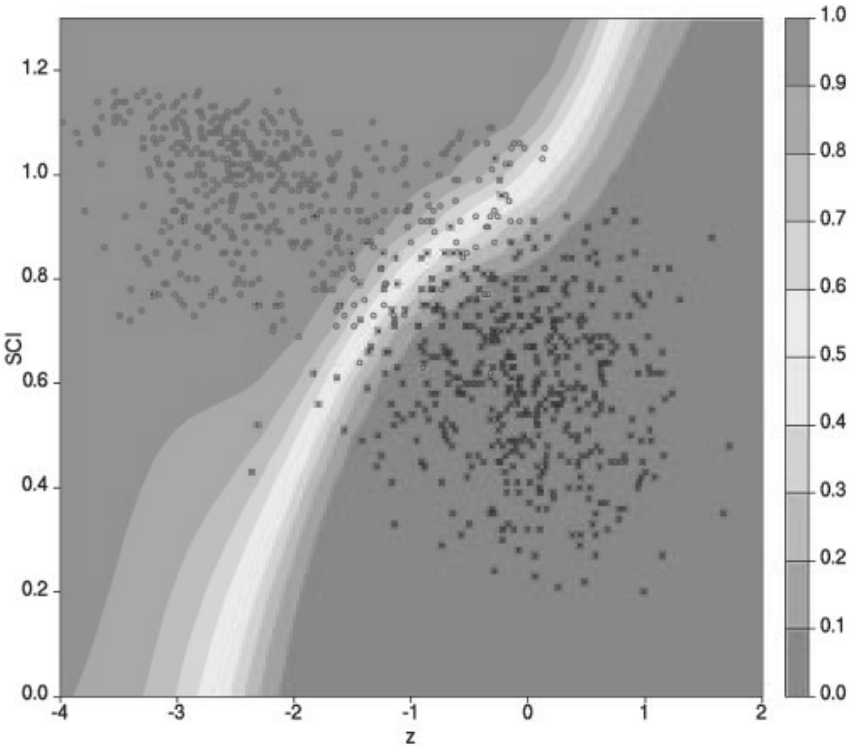
Back to Paper: Classification SVM

- Binary classification SVM trained to classify alignments as “RNA” or “other”
- Classification parameters were:
  - Mean of MFE z scores of the individual sequences
  - SCI
  - Mean pairwise identity
  - Number of sequences in the alignment

Information content of multiple alignment depends strongly on pairwise identity and number of sequences

- Training data
  - All classes of ncRNA with exception of tmRNAs and U70 small nucleolar RNAs
  - For each native alignment, included one randomized version
- Testing
  - Generated models from all classes, leaving out one class at a time
  - Alignments with mean pairwise identities between 50-100%
- Kernel function
  - Radial basis function $K(\mathbf{x},\mathbf{x}) = \exp(-\gamma \| \mathbf{x} - \mathbf{x} \|^2)$, with $\gamma = 2$
  - Slack penalty variable C = 32

Resulting ncRNA Classification



- Alignments of tRNAs and 5S rRNAs with 2-4 sequences per alignment and mean pairwise identities between 60-90%
- Green circles – native alignments
- Red crosses – shuffled random controls
- Background cloror indicates RNA class probability in z-SCI plane

Results of RNAz

- At cutoff of classification probability (P) of 0.9 over 12 ncRNA types:
  - Average sensitivity = 72.27%
  - Average specificity = 98.93%
- Results varied by ncRNA type:
  - U70 snoRNA – stable but not well conserved
  - tmRNA – conserved, but not stable
- Scan of Comparative Regulatory Genomics (CORG) database:
  - 89 ncRNA regions with P > 0.5
  - 11 known ncRNAs; 78 unknown
  - Hits in 5' UTRs of protein coding genes, introns, unannotated regions

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