For written notes on this lecture, please read Chapters 4 and 7 of *The Practical Bioinformatician*, and Koh & Wong, “Recognition of Polyadenylation Sites from Arabidopsis Genomic Sequences”, *Proc GIW 2007*, pages 73--82
Plan

1. Transcription

2. Splicing

3. Translation

DNA → Primary transcript → mRNA → Protein
Some relevant biology
Central dogma

Replication → Transcription → Translation

DNA → RNA → Protein

...AATGGTACCGATGACCTG...

...AAUGGUACCGAUGACCUGGAGC...

...TRLRPLLALLALWP...
Players in protein synthesis
Transcription

• Synthesize mRNA from one strand of DNA
  – An enzyme RNA polymerase temporarily separates double-stranded DNA
  – It begins transcription at transcription start site
  – A → A, C→C, G→G, & T→U
  – Once RNA polymerase reaches transcription stop site, transcription stops

• Additional “steps” for Eukaryotes
  – Transcription produces pre-mRNA that contains both introns & exons
  – 5’ cap & poly-A tail are added to pre-mRNA
  – RNA splicing removes introns & mRNA is made
  – mRNA are transported out of nucleus
Translation

- Synthesize protein from mRNA
- Each amino acid is encoded by consecutive seq of 3 nucleotides, called a codon
- The decoding table from codon to amino acid is called genetic code

- $4^3 = 64$ diff codons
  $\Rightarrow$ Codons are not 1-to-1 corr to 20 amino acids
- All organisms use the same decoding table (except some mitochondrial genes)
- Amino acids can be classified into 4 groups. A single-base change in a codon is usu insufficient to cause a codon to code for an amino acid in diff group
## Genetic code

- **Start codon**
  - ATG (code for M)

- **Stop codon**
  - TAA
  - TAG
  - TGA

<table>
<thead>
<tr>
<th>First Position</th>
<th>Second Position of Codon</th>
<th>Third Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>TTT Phe [F]</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>TTC Phe [F]</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>TTA Leu [L]</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>TTG Leu [L]</td>
<td>G</td>
</tr>
<tr>
<td>C</td>
<td>CTT Leu [L]</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>CTC Leu [L]</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>CTA Leu [L]</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>CGT Leu [L]</td>
<td>G</td>
</tr>
<tr>
<td>A</td>
<td>ATT Ile [I]</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>ATC Ile [I]</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>ATA Ile [I]</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>ATG Met [M]</td>
<td>G</td>
</tr>
<tr>
<td>G</td>
<td>GGT Val [V]</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>GTC Val [V]</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>GTA Val [V]</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>GTG Val [V]</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>GCT Ala [A]</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>GCC Ala [A]</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>GCA Ala [A]</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>GCG Ala [A]</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>GAT Asp [D]</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>GAC Asp [D]</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>GAA Glu [E]</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>GAG Glu [E]</td>
<td>G</td>
</tr>
<tr>
<td></td>
<td>GGT Gly [G]</td>
<td>T</td>
</tr>
<tr>
<td></td>
<td>GGC Gly [G]</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>GGA Gly [G]</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>GGG Gly [G]</td>
<td>G</td>
</tr>
</tbody>
</table>
Example of computational translation - notice the indication of (alternative) start-codons:

VIRTUAL RIBOSOME

Translation table: Standard SGCO

>Seq1
Reading frame: 1

```
M V L S A A D K G N V K A A W G K V G G H A A E Y G A E A L
5' ATG GTG CTG TCT GCG CCG ACA AGG GA ATG TCA AGG GCG CTC TTG GG GCA AGG TTG GCG GCC ACC GC GCT GCA GAT TAT GCG CAG AGG GCC CTC TG 90

...>><...)))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))

E R M F L S F P T T K T Y F P H F D L S H G S A Q V K G H G
5' GAG AGG GAT GTT CTT GAG CT TCCC ACC ACC AAG ACC CTT ACT CT TCCC ACT TC TTG GAC CT GAG GCC AC GGT CCG CGC GAG GTCA AGG GG GCC ACG GC 180

...>><...)))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))

A K V A A A L T K A V E H L D D L P G A L S E L S D L H A H
5' GCC AGG TG GGC CC GCG CTC TCA AAG ACC GTG GAA CAC TGG ACG CAC TAG GCT GCG GCT GTG TCA GAT TAG GAC CTT GAC GCT GC CAC 270

...>><...)))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))

K L R V D F V N F K L L S H S L L V T L A S H L P S D F T F
5' AAG CTG CCG GTG GAC CCG GTC AACT TCA AGCT TTG AG CAC TCG CTG TGT GAG GCC ACT GCT GCC TCC ACC GCT GCC AAT TTT CAC CCC 360

...>><...)))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))

A V H A S L D K F L A N V S T V L T S K Y R *
5' GCC GT CAC GCT CCC TTG GAC AAG GTT CTT TG GCC AAG CTC GTG GAC CTT CAA ATAC CG TAA 429

...>><...)))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))))
```

Annotation key:

>>> : START codon (strict)

)))) : START codon (alternative)

*** : STOP
Translation initiation sites

An introduction to the World’s simplest TIS recognition system
Translation initiation site

DNA: A, C, G, T
mRNA: A, C, G, U

5′flank  exon  intron  exon  intron  exon  3′flank

transcription

coding region

Ribosome

The ribosome reads the sequence 3 nucleotides at a time. Each group of 3 nucleotides is a single codon.
A sample cDNA

• What makes the second ATG the TIS?
Approach

• Training data gathering

• Signal generation
  – k-grams, distance, domain know-how, ...

• Signal selection
  – Entropy, $\chi^2$, CFS, t-test, domain know-how...

• Signal integration
  – SVM, ANN, PCL, CART, C4.5, kNN, ...
Training & testing data

- Vertebrate dataset of Pedersen & Nielsen [ISMB'97]
- 3312 sequences
- 13503 ATG sites
- 3312 (24.5%) are TIS
- 10191 (75.5%) are non-TIS
- Use for 3-fold x-validation expts
Signal generation

- **K-grams (ie., k consecutive letters)**
  - $K = 1, 2, 3, 4, 5, \ldots$
  - Window size vs. fixed position
  - Up-stream, downstream vs. any where in window
  - In-frame vs. any frame
### Signal generation: Example

**Exercise #1**

<table>
<thead>
<tr>
<th>Position</th>
<th>Sequence</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>299</td>
<td>CGTGCGAGCTGCCCAGCCATGCAGCTGAACACTGACTCCAGCTGTG</td>
<td>80</td>
</tr>
<tr>
<td>160</td>
<td>CCCAGGGCTTCAAAGACTTCTCAGCTGAGCATGCTTTTGCTGTCAAGGCAGCTGTA</td>
<td>160</td>
</tr>
<tr>
<td>240</td>
<td>GGAGGCAGATGAGAAGGGAAGTGCCCTGGAGGAAGGAAAGGCGCTGTCGGGAGGA</td>
<td>240</td>
</tr>
<tr>
<td>320</td>
<td>CCTCTCGGCCCAGGACTTCTCCAGGACAAAGCCCTCACCACCACCAAGGACTCCCCT</td>
<td>320</td>
</tr>
</tbody>
</table>

- **Window = ±100 bases**
- **In-frame, downstream**
  - GCT = 1, TTT = 1, ATG = 1...
- **Any-frame, downstream**
  - GCT = 3, TTT = 2, ATG = 2...
- **In-frame, upstream**
  - GCT = 2, TTT = 0, ATG = 0, ...

**Exercise:** Find the in-frame downstream ATG

**Exercise:** What are the possible k-grams (k=3) in this sequence?
Feature generation - Summary

Raw Data

206 BBCALCB.1 CAT X71666 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
CCGTCAGAGCAGCAGCCGACACTCTTTCTCTGCTGCGAGCCGGAGCCGGCAGCCGCAAATGGGAAATGAGGCAAGTATCT
TTGGAAATGTGCTCACACTTTTGATGCAAGATGAAATTTAAGGGTGAGGAAGAGATTTAAGAAGGCTCGATTTGGACAATTTC
TGGTTCTTTGAGTGCGGAGAAGGTTCATGTCTCTCACTCTGAGTTACAA

An ATG segment – positive sample

A feature vector --- upstream/downstream inframe 3 grams

1,0,0,0,1,0,0,0,1,2,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1,0,2,0,2,1,0,0,0,1,0,0,0,0,0,0,0,0,0,0,2,0,
0,0,0,0,0,1,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,
Too many features

- For each value of k, there are $4^k \times 3 \times 2^k$ k-grams

- If we use $k = 1, 2, 3, 4, 5$, we have $24 + 96 + 384 + 1536 + 6144 = 8184$ features!

- This is too many for most machine learning algorithms
Signal selection: Basic idea

- Choose a signal with low intra-class distance
- Choose a signal with high inter-class distance

Which of these three features are best for distinguishing Class 1 from Class 2? Why?

Exercise #2
Signal selection: t-statistics

The t-stats of a signal is defined as

\[ t = \frac{|\mu_1 - \mu_2|}{\sqrt{\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}}} \]

where \( \sigma_i^2 \) is the variance of that signal in class \( i \), \( \mu_i \) is the mean of that signal in class \( i \), and \( n_i \) is the size of class \( i \).
Signal selection: “MIT-correlation

The MIT-correlation value of a signal is defined as

\[ MIT = \frac{|\mu_1 - \mu_2|}{\sigma_1 + \sigma_2} \]

where \( \sigma_i \) is the standard deviation of that signal in class \( i \) and \( \mu_i \) is the mean of that signal in class \( i \).
Signal selection: $\chi^2$

The $\chi^2$ value of a signal is defined as:

$$\chi^2 = \sum_{i=1}^{m} \sum_{j=1}^{k} \frac{(A_{ij} - E_{ij})^2}{E_{ij}},$$

where $m$ is the number of intervals, $k$ the number of classes, $A_{ij}$ the number of samples in the $i$th interval, $j$th class, $R_i$ the number of samples in the $i$th interval, $C_j$ the number of samples in the $j$th class, $N$ the total number of samples, and $E_{ij}$ the expected frequency of $A_{ij}$ ($E_{ij} = R_i * C_j / N$).
Example

- Suppose you have a sample of 50 men and 50 women and the following weight distribution is observed:

<table>
<thead>
<tr>
<th></th>
<th>obs</th>
<th>exp</th>
<th>(obs – exp)^2/exp</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM</td>
<td>40</td>
<td>60*50/100=30</td>
<td>3.3</td>
</tr>
<tr>
<td>HW</td>
<td>20</td>
<td>60*50/100=30</td>
<td>3.3</td>
</tr>
<tr>
<td>LM</td>
<td>10</td>
<td>40*50/100=20</td>
<td>5.0</td>
</tr>
<tr>
<td>LW</td>
<td>30</td>
<td>40*50/100=20</td>
<td>5.0</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 16.6 \]

P = 0.00004, df = 1
So weight and sex are not indep

- Is weight a good attribute for distinguishing men from women?

Exercise #3
Signal selection: CFS

• Instead of scoring individual signals, how about scoring a group of signals as a whole?

• CFS
  – Correlation-based Feature Selection
  – A good group contains signals that are highly correlated with the class, and yet uncorrelated with each other

• What is the main challenge in implementing CFS?
Distributions of two 3-grams

\[ \chi^2 = 1672.97447 \]

\[ \chi^2 = 0 \]

- Which is the better one? Why?

Exercise #4
Sample k-grams selected by CFS for recognizing TIS

Kozak consensus

- Position –3
- in-frame upstream ATG
- in-frame downstream
  - TAA, TAG, TGA,
  - CTG, GAC, GAG, and GCC

Leaky scanning

Stop codon

Codon bias?
Signal integration

• kNN
  – Given a test sample, find the k training samples that are most similar to it. Let the majority class win

• SVM
  – Given a group of training samples from two classes, determine a separating plane that maximises the margin of error

• Naïve Bayes, ANN, C4.5, ...
## Results: 3-fold x-validation

### Table: Classification Accuracy

<table>
<thead>
<tr>
<th>Method</th>
<th>(\frac{TP}{TP+FN})</th>
<th>(\frac{TN}{TN+FP})</th>
<th>(\frac{TP}{TP+FP})</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve Bayes</td>
<td>84.3%</td>
<td>86.1%</td>
<td>66.3%</td>
<td>85.7%</td>
</tr>
<tr>
<td>SVM</td>
<td>73.9%</td>
<td>93.2%</td>
<td>77.9%</td>
<td>88.5%</td>
</tr>
<tr>
<td>Neural Network</td>
<td>77.6%</td>
<td>93.2%</td>
<td>78.8%</td>
<td>89.4%</td>
</tr>
<tr>
<td>Decision Tree</td>
<td>74.0%</td>
<td>94.4%</td>
<td>81.1%</td>
<td>89.4%</td>
</tr>
</tbody>
</table>

### Exercise:

What is \(\frac{TP}{TP+FP}\)?

The table above shows the results of a 3-fold cross-validation test for different classification models. The accuracy of each model is calculated based on the ratio of true positives (TP) to the total number of actual positives (TP + FN) in the test set. The table includes Naïve Bayes, SVM, Neural Network, and Decision Tree models, with their corresponding accuracies.
Improvement by voting

- Apply any 3 of Naïve Bayes, SVM, Neural Network, & Decision Tree. Decide by majority

<table>
<thead>
<tr>
<th>Model Configuration</th>
<th>TP/(TP + FN)</th>
<th>TN/(TN + FP)</th>
<th>TP/(TP + FP)</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB+SVM+NN</td>
<td>79.2%</td>
<td>92.1%</td>
<td>76.5%</td>
<td>88.9%</td>
</tr>
<tr>
<td>NB+SVM+Tree</td>
<td>78.8%</td>
<td>92.0%</td>
<td>76.2%</td>
<td>88.8%</td>
</tr>
<tr>
<td>NB+NN+Tree</td>
<td>77.6%</td>
<td>94.5%</td>
<td>82.1%</td>
<td>90.4%</td>
</tr>
<tr>
<td>SVM+NN+Tree</td>
<td>75.9%</td>
<td>94.3%</td>
<td>81.2%</td>
<td>89.8%</td>
</tr>
<tr>
<td>Best of 4</td>
<td>84.3%</td>
<td>94.4%</td>
<td>81.1%</td>
<td>89.4%</td>
</tr>
<tr>
<td>Worst of 4</td>
<td>73.9%</td>
<td>86.1%</td>
<td>66.3%</td>
<td>85.7%</td>
</tr>
</tbody>
</table>
**Improvement by scanning**

- Apply Naïve Bayes or SVM left-to-right until first ATG predicted as positive. That’s the TIS
- Naïve Bayes & SVM models were trained using TIS vs. Up-stream ATG

<table>
<thead>
<tr>
<th></th>
<th>TP/(TP + FN)</th>
<th>TN/(TN + FP)</th>
<th>TP/(TP + FP)</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB</td>
<td>84.3%</td>
<td>86.1%</td>
<td>66.3%</td>
<td>85.7%</td>
</tr>
<tr>
<td>SVM</td>
<td>73.9%</td>
<td>93.2%</td>
<td>77.9%</td>
<td>88.5%</td>
</tr>
<tr>
<td>NB+Scanning</td>
<td>87.3%</td>
<td>96.1%</td>
<td>87.9%</td>
<td>93.9%</td>
</tr>
<tr>
<td>SVM+Scanning</td>
<td>88.5%</td>
<td>96.3%</td>
<td>88.6%</td>
<td>94.4%</td>
</tr>
</tbody>
</table>
## Performance comparison

<table>
<thead>
<tr>
<th>Method</th>
<th>TP/(TP + FN)</th>
<th>TN/(TN + FP)</th>
<th>TP/(TP + FP)</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB</td>
<td>84.3%</td>
<td>86.1%</td>
<td>66.3%</td>
<td>85.7%</td>
</tr>
<tr>
<td>Decision Tree</td>
<td>74.0%</td>
<td>94.4%</td>
<td>81.1%</td>
<td>89.4%</td>
</tr>
<tr>
<td>NB+NN+Tree</td>
<td>77.6%</td>
<td>94.5%</td>
<td>82.1%</td>
<td>90.4%</td>
</tr>
<tr>
<td>SVM+Scanning</td>
<td>88.5%</td>
<td>96.3%</td>
<td>88.6%</td>
<td>94.4%*</td>
</tr>
<tr>
<td>Pedersen&amp;Nielsen</td>
<td>78%</td>
<td>87%</td>
<td>-</td>
<td>85%</td>
</tr>
<tr>
<td>Zien</td>
<td>69.9%</td>
<td>94.1%</td>
<td>-</td>
<td>88.1%</td>
</tr>
<tr>
<td>Hatzigeorgiou</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>94%*</td>
</tr>
</tbody>
</table>

* result not directly comparable
Technique comparison

• **Pedersen&Nielsen [ISMB’97]**
  - Neural network
  - No explicit features

• **Zien [Bioinformatics’00]**
  - SVM+kernel engineering
  - No explicit features

• **Hatzigeorgiou [Bioinformatics’02]**
  - Multiple neural networks
  - Scanning rule
  - No explicit features

• **Our approach**
  - Explicit feature generation
  - Explicit feature selection
  - Use any machine learning method w/o any form of complicated tuning
  - Scanning rule is optional
mRNA $\rightarrow$ protein

How about using k-grams from the translation?

<table>
<thead>
<tr>
<th>First</th>
<th>U</th>
<th>C</th>
<th>A</th>
<th>G</th>
<th>Last</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>Phe</td>
<td>Ser</td>
<td>Tyr</td>
<td>Cys</td>
<td>C</td>
</tr>
<tr>
<td>Phe</td>
<td>Ser</td>
<td>Tyr</td>
<td>Cys</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Ser</td>
<td>Stop (Ochre)</td>
<td>Stop (Umbre)</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Ser</td>
<td>Stop (Amber)</td>
<td>Trp</td>
<td>G</td>
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</tr>
<tr>
<td>C</td>
<td>Leu</td>
<td>Pro</td>
<td>His</td>
<td>Arg</td>
<td>U</td>
</tr>
<tr>
<td>Leu</td>
<td>Pro</td>
<td>His</td>
<td>Arg</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Pro</td>
<td>Gln</td>
<td>Arg</td>
<td>A</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>Pro</td>
<td>Gln</td>
<td>Arg</td>
<td>G</td>
<td></td>
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<tr>
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<td>Thr</td>
<td>Asn</td>
<td>Ser</td>
<td>C</td>
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<td>Ile</td>
<td>Thr</td>
<td>Lys</td>
<td>Arg</td>
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<td>Thr</td>
<td>Lys</td>
<td>Arg</td>
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<td>Val</td>
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<td>Asp</td>
<td>Gly</td>
<td>U</td>
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<td>Ala</td>
<td>Asp</td>
<td>Gly</td>
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<td>Ala</td>
<td>Glu</td>
<td>Gly</td>
<td>A</td>
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</tr>
<tr>
<td>Val</td>
<td>Ala</td>
<td>Glu</td>
<td>Gly</td>
<td>G</td>
<td></td>
</tr>
</tbody>
</table>

Exercise: List the first 10 amino acid in our example sequence
Amino-acid features

```
......GGACGGATGACTGCC......CTCGATA TGGCACCT......TGCTAA ATGACAAATA......
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# Amino-acid features

![Diagram of amino-acid features](image)

**New feature space (total of 927 features + class label)**

<table>
<thead>
<tr>
<th>42 1-gram amino acid patterns</th>
<th>882 2-gram amino acid patterns</th>
<th>3 bio-knowledge patterns</th>
<th>class label</th>
</tr>
</thead>
</table>

**Frequency as values**

| 1, 3, 5, 0, 4, ... | 6, 2, 7, 0, 5, ... | N, N, N, | False |
| 6, 5, 7, 9, 0, ... | 2, 0, 3, 10, 0, ... | Y, Y, Y, | True |
Amino acid K-grams discovered by entropy

- Position -3
- in-frame upstream ATG
- in-frame downstream
  - TAA, TAG, TGA,
  - CTG, GAC, GAG, and GCC

<table>
<thead>
<tr>
<th>Fold</th>
<th>UP-ATG</th>
<th>DOWN-STOP</th>
<th>UP3-AorG</th>
<th>DOWN-A</th>
<th>DOWN-V</th>
<th>UP-A</th>
<th>DOWN-L</th>
<th>DOWN-D</th>
<th>DOWN-E</th>
<th>UP-G</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>9</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>7</td>
<td>10</td>
</tr>
</tbody>
</table>
Independent validation sets

• **A. Hatzigeorgiou:**
  – 480 fully sequenced human cDNAs
  – 188 left after eliminating sequences similar to training set (Pedersen & Nielsen’s)
  – 3.42% of ATGs are TIS

• **Our own:**
  – Well-characterized human gene sequences from chromosome X (565 TIS) and chromosome 21 (180 TIS)
Validation results, on Hatzigeorgiou

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Precision</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>SVMs(linear)</td>
<td>96.28%</td>
<td>89.15%</td>
<td>25.31%</td>
<td>89.42%</td>
</tr>
<tr>
<td>SVMs(quad)</td>
<td>94.14%</td>
<td>90.13%</td>
<td>26.70%</td>
<td>90.28%</td>
</tr>
<tr>
<td>Ensemble Trees</td>
<td>92.02%</td>
<td>92.71%</td>
<td>32.52%</td>
<td>92.68%</td>
</tr>
<tr>
<td>SVMs(quad)</td>
<td>85.21%</td>
<td>89.74%</td>
<td>34.68%</td>
<td>89.02%</td>
</tr>
</tbody>
</table>

- Using top 100 features selected by entropy and trained on Pedersen & Nielsen’s dataset
Validation results, on Chr X & 21

- Using top 100 features selected by entropy and trained on Pedersen & Nielsen’s
About the inventor: Huiqing Liu

- **Huiqing Liu**
  - PhD, NUS, 2004
  - Currently PI at Incyte
  - Asian Innovation Gold Award 2003
  - New Jersey Cancer Research Award for Scientific Excellence 2008
  - Gallo Prize 2008
Recognition of Transcription Start Sites

An introduction to the World’s best TSS recognition system:
A heavy tuning approach

NUS
National University of Singapore
Transcription start site

DNA: A, C, G, T

mRNA: A, C, G, U

coding region
Structure of Dragon Promoter Finder

-200 to +50 window size

Model selected based on desired sensitivity
Each model has two submodels based on GC content

Exercise: Why are the submodels based on GC content?

$$\frac{(C+G)}{\text{Window Size}} = \frac{\#C + \#G}{\text{Window Size}}$$
Data analysis within submodel

K-gram (k = 5) positional weight matrix
Promoter, exon, intron sensors

- These sensors are positional weight matrices of k-grams, \( k = 5 \) (aka pentamers)
- They are calculated as below using promoter, exon, intron data respectively

\[
\sigma = \frac{\sum_{i=1}^{L-4} p_j^i \otimes f_{j,i}}{\sum_{i=1}^{L-4} \max_j f_{j,i}},
\]

where

\[
p_j^i \otimes f_{j,i} = \begin{cases} 
  f_{j,i}, & \text{if } p_i = p_j^i \\
  0, & \text{if } p_i \neq p_j^i 
\end{cases}
\]

- Pentamer at \( i^{th} \) position in input
- \( j^{th} \) pentamer at \( i^{th} \) position in training window
- Window size
- Frequency of \( j^{th} \) pentamer at \( i^{th} \) position in training window
Just to make sure you know what I mean …

- Give me 3 DNA seq of length 10:
  - Seq_1 = ACCGAGTTCT
  - Seq_2 = AGTGTACCTG
  - Seq_3 = AGTTCGTATG

- Then

<table>
<thead>
<tr>
<th>1-mer</th>
<th>pos1</th>
<th>pos2</th>
<th>pos3</th>
<th>pos4</th>
<th>pos5</th>
<th>pos6</th>
<th>pos7</th>
<th>pos8</th>
<th>pos9</th>
<th>pos10</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3/3</td>
<td>0/3</td>
<td>0/3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0/3</td>
<td>1/3</td>
<td>1/3</td>
<td></td>
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<tr>
<td>G</td>
<td>0/3</td>
<td>2/3</td>
<td>0/3</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>T</td>
<td>0/3</td>
<td>0/3</td>
<td>2/3</td>
<td></td>
<td></td>
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</tbody>
</table>

Exercise #5: Fill in the rest of the table
Just to make sure you know what I mean …

• **Give me 3 DNA seq of length 10:**
  – Seq$_1$ = ACCGAGTTCT
  – Seq$_2$ = AGTGTACCTG
  – Seq$_3$ = AGTTCGTATG

• **Then**

<table>
<thead>
<tr>
<th>2-mer</th>
<th>pos1</th>
<th>pos2</th>
<th>pos3</th>
<th>pos4</th>
<th>pos5</th>
<th>pos6</th>
<th>pos7</th>
<th>pos8</th>
<th>pos9</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>1/3</td>
<td>0/3</td>
<td>0/3</td>
<td></td>
<td></td>
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<tr>
<td>TT</td>
<td>0/3</td>
<td>0/3</td>
<td>1/3</td>
<td></td>
<td></td>
<td></td>
<td>1/3</td>
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</tbody>
</table>

Exercise: How many rows should this 2-mer table have? How many rows should the pentamer table have?

Exercise: Fill in the rest of the table
Data preprocessing & ANN

Tuning parameters

\[ s_E = sat(\sigma_p - \sigma_e, a_e, b_e), \]
\[ s_I = sat(\sigma_p - \sigma_i, a_i, b_i), \]
\[ s_{EI} = sat(\sigma_e - \sigma_i, a_{ei}, b_{ei}), \]
where the function \( sat \) is defined by

\[ sat(x, a, b) = \begin{cases} 
  a, & \text{if } x > a \\
  x, & \text{if } b \leq x \leq a. \\
  b, & \text{if } b > x
\end{cases} \]

Simple feedforward ANN trained by the Bayesian regularisation method

\[ \text{tanh}(x) = \frac{e^x - e^{-x}}{e^x + e^{-x}} \]

\[ \text{net} = \sum s_i \ast w_i \]
Accuracy comparison

Accuracy of Dragon Promoter Finder Ver. 1.2 & 1.3

Positive predictive value ppv in % = 100 x TP/(TP+FP)

Sensitivity in % = 100 x TP/(TP+FN)

- DPF v1.2
- DPF v1.3
- NNPP2.1 (0.99)
- NNPP2.1 (0.8)
- PromoterInspector
- Promoter2.0

with C+G submodels
without C+G submodels
Training data criteria & preparation

- Contain both positive and negative sequences
- Sufficient diversity, resembling different transcription start mechanisms
- Sufficient diversity, resembling different non-promoters
- Sanitized as much as possible

- TSS taken from
  - 793 vertebrate promoters from EPD
  - -200 to +50 bp of TSS

- non-TSS taken from
  - GenBank,
  - 800 exons
  - 4000 introns,
  - 250 bp,
  - non-overlapping,
  - <50% identities
Tuning data preparation

- To tune adjustable system parameters in Dragon, we need a separate tuning data set

- **TSS taken from**
  - 20 full-length gene seqs with known TSS
  - -200 to +50 bp of TSS
  - no overlap with EPD

- **Non-TSS taken from**
  - 1600 human 3’UTR seqs
  - 500 human exons
  - 500 human introns
  - 250 bp
  - no overlap
Testing data criteria & preparation

- Seqs should be from the training or evaluation of other systems (no bias!)
- Seqs should be disjoint from training and tuning data sets
- Seqs should have TSS
- Seqs should be cleaned to remove redundancy, <50% identities

- 159 TSS from 147 human and human virus seqs
- Cumulative length of more than 1.15Mbp
- Taken from GENESCAN, Geneld, Genie, etc.
About the inventor: Vlad Bajic

• **Vladimir B. Bajic**
  – Principal Scientist, I²R, 2001-2006
  – Currently Director & Professor, Computational Bioscience Research Center, KAUST
Recognition of Poly-A signal sites

A twist to the “feature generation, feature selection, feature integration” approach
Eukaryotic pre-mRNA processing

[Diagram showing the process of pre-mRNA processing, including steps like capping/splicing, cleavage, and polyadenylation, with images of exons, introns, 5'UTR, and 3'UTR.]
Polyadenylation in eukaryotes

- **Addition of poly(A) tail to RNA**
  - Begins as transcription finishes
  - 3’-most segment of newly-made RNA is cleaved off
  - Poly(A) tail is then synthesized at 3' end

- **Poly(A) tail is imp't for nuclear export, translation & stability of mRNA**

- **Tail is shortened over time. When short enough, the mRNA is degraded**
### Poly-A signals in human (Gautheret et al., 2000)

Table 2. Most Significant Hexamers in 3’ Fragments: Clustered Hexamers

<table>
<thead>
<tr>
<th>Hexamer</th>
<th>Observed (expected)</th>
<th>% sites</th>
<th>$p^b$</th>
<th>Position average ± SD</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAUAAA</td>
<td>3286 (317)</td>
<td>58.2</td>
<td>0</td>
<td>-16 ± 4.7</td>
<td>-45 -35</td>
</tr>
<tr>
<td>AUUAAA</td>
<td>843 (112)</td>
<td>14.9</td>
<td>0</td>
<td>-17 ± 5.3</td>
<td>-25 -15</td>
</tr>
<tr>
<td>AGUAAA</td>
<td>156 (32)</td>
<td>2.7</td>
<td>$6 \times 10^{-57}$</td>
<td>-16 ± 5.9</td>
<td>-5</td>
</tr>
<tr>
<td>UAUAAA</td>
<td>180 (53)</td>
<td>3.2</td>
<td>$4 \times 10^{-45}$</td>
<td>-18 ± 7.8</td>
<td>0</td>
</tr>
<tr>
<td>CAUAAA</td>
<td>76 (23)</td>
<td>1.3</td>
<td>$1 \times 10^{-16}$</td>
<td>-17 ± 5.9</td>
<td>0</td>
</tr>
<tr>
<td>GAUAAA</td>
<td>72 (21)</td>
<td>1.3</td>
<td>$2 \times 10^{-16}$</td>
<td>-18 ± 6.9</td>
<td>0</td>
</tr>
<tr>
<td>AAUAUA</td>
<td>96 (33)</td>
<td>1.7</td>
<td>$2 \times 10^{-19}$</td>
<td>-18 ± 6.9</td>
<td>0</td>
</tr>
<tr>
<td>AAUACA</td>
<td>70 (16)</td>
<td>1.2</td>
<td>$5 \times 10^{-23}$</td>
<td>-18 ± 8.7</td>
<td>0</td>
</tr>
<tr>
<td>AAUAGA</td>
<td>43 (14)</td>
<td>0.7</td>
<td>$1 \times 10^{-9}$</td>
<td>-18 ± 6.3</td>
<td>0</td>
</tr>
<tr>
<td>AAAAAG</td>
<td>49 (11)</td>
<td>0.8</td>
<td>$5 \times 10^{-17}$</td>
<td>-18 ± 8.9</td>
<td>0</td>
</tr>
<tr>
<td>ACUAAA</td>
<td>36 (11)</td>
<td>0.6</td>
<td>$1 \times 10^{-06}$</td>
<td>-17 ± 8.1</td>
<td>0</td>
</tr>
<tr>
<td>AAGAAAA</td>
<td>62 (10)</td>
<td>1.1</td>
<td>$9 \times 10^{-28}$</td>
<td>-19 ± 11</td>
<td>0</td>
</tr>
<tr>
<td>AAUGAAA</td>
<td>49 (10)</td>
<td>0.8</td>
<td>$4 \times 10^{-18}$</td>
<td>-20 ± 10</td>
<td>0</td>
</tr>
<tr>
<td>UUUAAA</td>
<td>69 (20)</td>
<td>1.2</td>
<td>$3 \times 10^{-18}$</td>
<td>-17 ± 12</td>
<td>0</td>
</tr>
<tr>
<td>AAAACA</td>
<td>29 (5)</td>
<td>0.5</td>
<td>$8 \times 10^{-12}$</td>
<td>-20 ± 10</td>
<td>0</td>
</tr>
<tr>
<td>GGGGCU</td>
<td>22 (3)</td>
<td>0.3</td>
<td>$9 \times 10^{-12}$</td>
<td>-24 ± 13</td>
<td>0</td>
</tr>
</tbody>
</table>
Poly-A signals in Arabidopsis

In contrast to human, PAS in Arab is highly degenerate. E.g., only 10% of Arab PAS is AAUAAA!
Approach on Arab PAS sites (I)

Legend:
- Feature Generation
- Feature Selection
- Feature Integration

Flow:
1. Training Data
2. Feature Generation
3. Feature Selection
4. Feature Integration
5. Test Data
6. Classification Model
7. Prediction scores at every 10 bp interval
8. Cascade Classifier (SMO2)
9. (+ve) if score > threshold
Approach on Arab PAS sites (II)

- **Data collection**
  - #1 from Hao Han, 811 +ve seq (-200/+200)
  - #2 from Hao Han, 9742 -ve seq (-200/+200)
  - #3 from Qingshun Li,
    - 6209 (+ve) seq (-300/+100)
    - 1581 (-ve) intron (-300/+100)
    - 1501 (-ve) coding (-300/+100)
    - 864 (-ve) 5’utr (-300/+100)

- **Feature generation**
  - 3-grams, compositional features (4U/1N, G/U*7, etc)
  - Freq of features above in 3 diff windows: (-110/+5), (-35/+15), (-50/+30)

- **Feature selection**
  - $\chi^2$

- **Feature integration & Cascade**
  - SVM
Score profile relative to candidate sites

![Score profile graph]

- **Ave Score**
- **Location**
- **(+ve)**
- **(-ve)**
Validation results

<table>
<thead>
<tr>
<th>SN_0</th>
<th>SMO 1</th>
<th>SMO 2</th>
<th>PASS 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Sequences</td>
<td>SN &amp; SP</td>
<td>Threshold</td>
<td>SN &amp; SP</td>
</tr>
<tr>
<td>CDS</td>
<td>90%</td>
<td>0.26</td>
<td>94%</td>
</tr>
<tr>
<td>5’UTR</td>
<td>79%</td>
<td>0.42</td>
<td>85%</td>
</tr>
<tr>
<td>Intron</td>
<td>64%</td>
<td>0.59</td>
<td>71%</td>
</tr>
</tbody>
</table>

Table 2. Equal-error-rate points of SMO1, SMO2, and PASS 1.0 for SN_10.

<table>
<thead>
<tr>
<th>SN_10</th>
<th>SMO 1</th>
<th>SMO 2</th>
<th>PASS 1.0</th>
</tr>
</thead>
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<tr>
<td>Control Sequences</td>
<td>SN &amp; SP</td>
<td>Threshold</td>
<td>SN &amp; SP</td>
</tr>
<tr>
<td>CDS</td>
<td>94%</td>
<td>0.36</td>
<td>96%</td>
</tr>
<tr>
<td>5’UTR</td>
<td>86%</td>
<td>0.53</td>
<td>89%</td>
</tr>
<tr>
<td>Intron</td>
<td>73%</td>
<td>0.68</td>
<td>77%</td>
</tr>
</tbody>
</table>

Table 2. Equal-error-rate points of SMO1, SMO2, and PASS 1.0 for SN_30.

<table>
<thead>
<tr>
<th>SN_30</th>
<th>SMO 1</th>
<th>SMO 2</th>
<th>PASS 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Sequences</td>
<td>SN &amp; SP</td>
<td>Threshold</td>
<td>SN &amp; SP</td>
</tr>
<tr>
<td>CDS</td>
<td>97%</td>
<td>0.44</td>
<td>97%</td>
</tr>
<tr>
<td>5’UTR</td>
<td>90%</td>
<td>0.62</td>
<td>92%</td>
</tr>
<tr>
<td>Intron</td>
<td>79%</td>
<td>0.75</td>
<td>83%</td>
</tr>
</tbody>
</table>
About the inventor: Koh Chuan Hock

- **Koh Chuan Hock**
  - BComp (CB), NUS, 2008
  - PhD, NUS, 2012
  - Currently Data Scientist at Indeed Inc, Japan
Concluding remarks…
What have we learned?

• Gene feature recognition applications
  – TIS, TSS, PAS

• General methodology
  – “Feature generation, feature selection, feature integration”

• Important tactics
  – Multiple models to optimize overall performance
  – Feature transformation (DNA → amino acid)
  – Classifier cascades
Any question?
Acknowledgements

• The slides for PAS site prediction are adapted from slides given to me by Koh Chuan Hock
References (TIS recognition)


- J. Li et al., “Techniques for Recognition of Translation Initiation Sites”, *The Practical Bioinformatician*, Chapter 4, pages 71—90, 2004
References (TSS recognition)


• M.Scherf et al., “Highly specific localisation of promoter regions in large genome sequences by PromoterInspector”, JMB 297:599--606, 2000

References (PAS recognition)


References (Feature selection)

