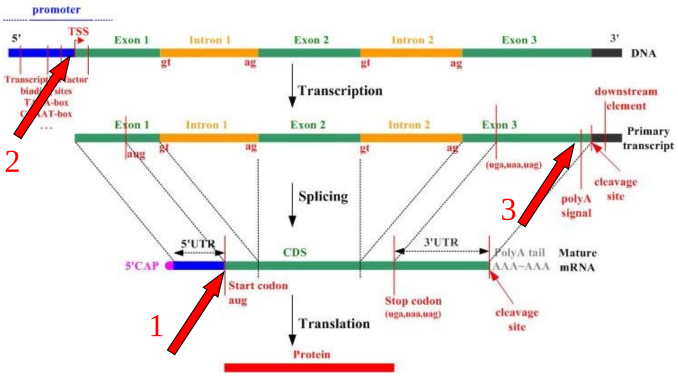
For written notes on this lecture, please read Chapters 4 and 7 of *The Practical Bioinformatician*, and Koh & Wong, “Recognition of Polyadenylation Sites from Arabidopsis Genomic Sequences”, *Proc GIW 2007*, pages 73--82

CS2220: Introduction to Computational Biology
Lecture 3: Gene Feature Recognition

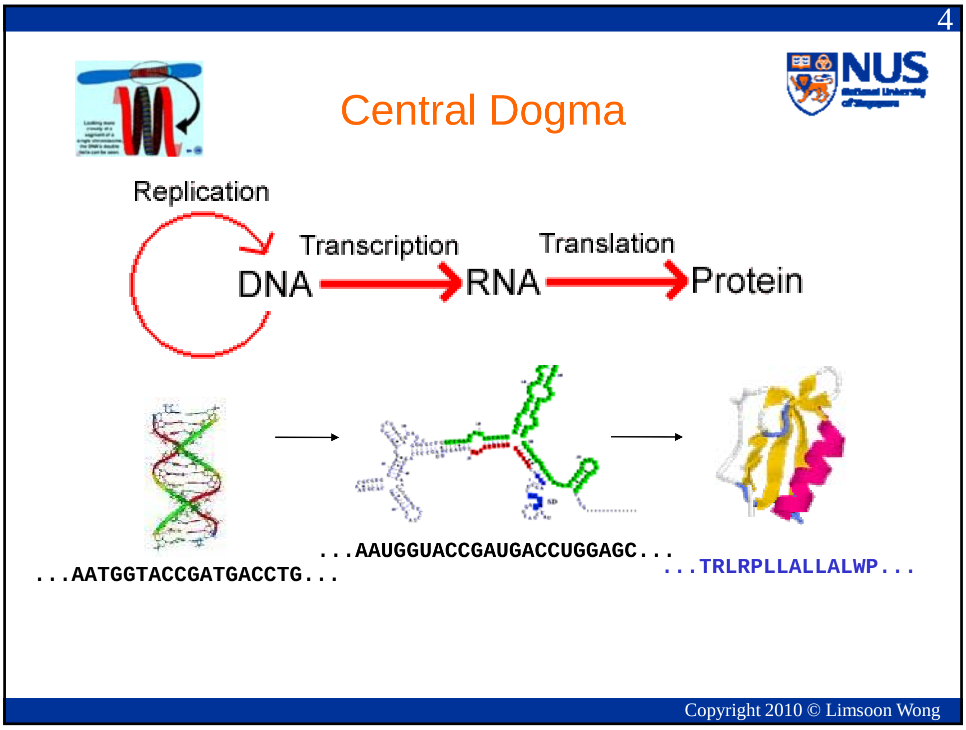
Limsoon Wong

28 January 2010

Plan



Some Relevant Biology



Central Dogma

Replication

Transcription

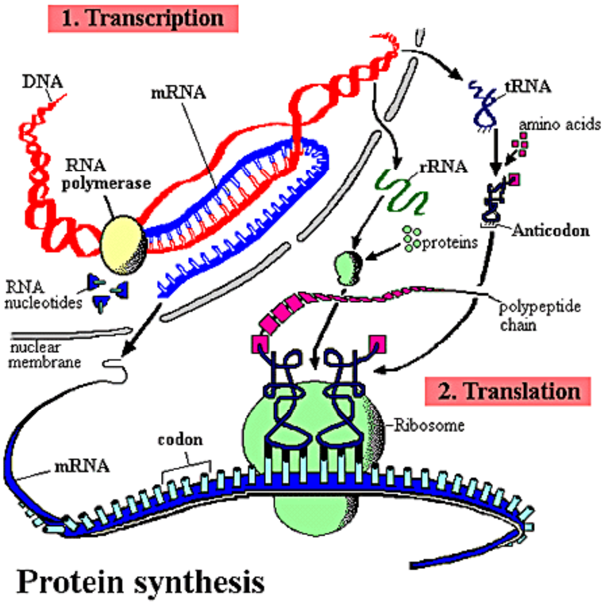
Translation

DNA → RNA → Protein

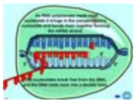
...AATGGTACCGATGACCTG...

...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**

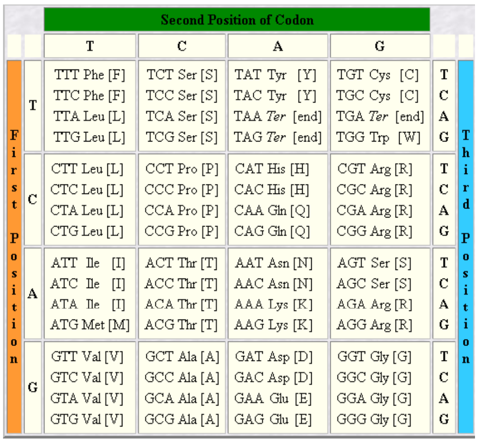
- **4^3=64 diff codons**

⇒ **Codons are not 1-to-1 corr to 20 amino acids**

- **All organisms use the same decoding table (except some mitochrondrial 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

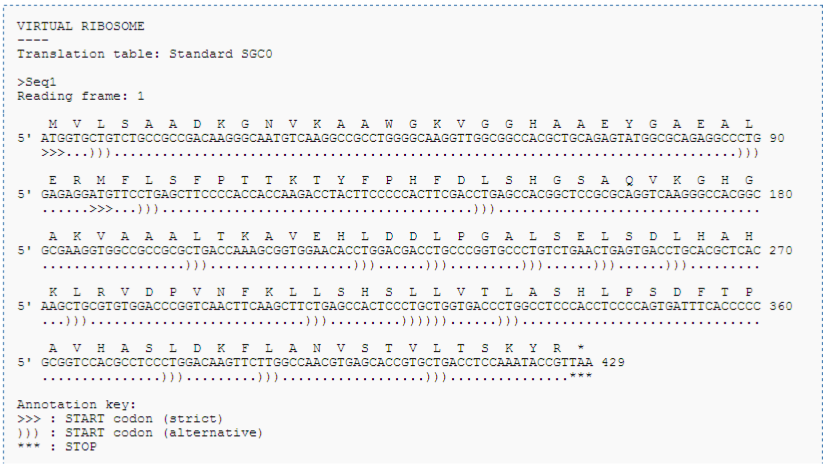


		Second Position of Codon				
		T	C	A	G	
First Position	T	TTT Phe [F]	TCT Ser [S]	TAT Tyr [Y]	TGT Cys [C]	T
		TTC Phe [F]	TCC Ser [S]	TAC Tyr [Y]	TGC Cys [C]	C
		TTA Leu [L]	TCA Ser [S]	TAA *Ter* [end]	TGA *Ter* [end]	A
		TTG Leu [L]	TCG Ser [S]	TAG *Ter* [end]	TGG Trp [W]	G
	C	CTT Leu [L]	CCT Pro [P]	CAT His [H]	CGT Arg [R]	T
		CTC Leu [L]	CCC Pro [P]	CAC His [H]	CGC Arg [R]	C
		CTA Leu [L]	CCA Pro [P]	CAA Gln [Q]	CGA Arg [R]	A
		CTG Leu [L]	CCG Pro [P]	CAG Gln [Q]	CGG Arg [R]	G
	A	ATT Ile [I]	ACT Thr [T]	AAT Asn [N]	AGT Ser [S]	T
		ATC Ile [I]	ACC Thr [T]	AAC Asn [N]	AGC Ser [S]	C
		ATA Ile [I]	ACA Thr [T]	AAA Lys [K]	AGA Arg [R]	A
		ATG Met [M]	ACG Thr [T]	AAG Lys [K]	AGG Arg [R]	G
	G	GTT Val [V]	GCT Ala [A]	GAT Asp [D]	GGT Gly [G]	T
		GTC Val [V]	GCC Ala [A]	GAC Asp [D]	GGC Gly [G]	C
		GTA Val [V]	GCA Ala [A]	GAA Glu [E]	GGA Gly [G]	A
		GTG Val [V]	GCG Ala [A]	GAG Glu [E]	GGG Gly [G]	G

Third Position

Example

Example of computational translation - notice the indication of (alternative) start-codons:

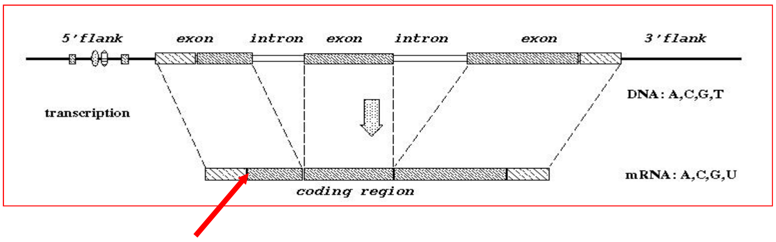


```
VIRTUAL RIBOSOME
----
Translation table: Standard SGC0

>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' ATGGTGCTGTCTGCCGCCGACAAGGGCAATGTCAAGGCCGCCTGGGGCAAGGTTGGCGGCCACGCTGCAGAGTATGGCGCAGAGGCCCTG 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' GAGAGGATGTTCCTGAGCTTCCCCACCACCAAGACCTACTTCCCCCACTTCGACCTGAGCCACGGCTCCGCGCAGGTCAAGGGCCACGGC 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' GCGAAGGTGGCCGCCGCGCTGACCAAAGCGGTGGAACACCTGGACGACCTGCCCGGTGCCCTGTCTGAACTGAGTGACCTGCACGCTCAC 270
   ..................)))..................)))......))).........)))......)))......))).........

    K  L  R  V  D  P  V  N  F  K  L  L  S  H  S  L  L  V  T  L  A  S  H  L  P  S  D  F  T  P
5' AAGCTGCGTGTGGACCCGGTCAACTTCAAGCTTCTGAGCCACTCCCTGCTGGTGACCCTGGCCTCCCACCTCCCCAGTGATTTCACCCCC 360
   ...))).............................))).........))))))......))).............................

    A  V  H  A  S  L  D  K  F  L  A  N  V  S  T  V  L  T  S  K  Y  R  *
5' GCGGTCCACGCCTCCCTGGACAAGTTCTTGGCCAACGTGAGCACCGTGCTGACCTCCAAATACCGTTAA 429
   ...............)))......... )))..................)))...............***

Annotation key:
>>> : START codon (strict)
))) : START codon (alternative)
*** : STOP
```

Recognition of Translation Initiation Sites

An introduction to the World’s simplest TIS recognition system

Translation Initiation Site



A Sample cDNA

```
 299 HSU27655.1 CAT U27655 Homo sapiens
CGTGTGTGCAGCAGCCTGCAGCTGCCCCAAGCC[illegible]GCTGAACACTGACTCCCAGCTGTG      80
CCCAGGGCTTCAAAGACTTCTCAGCTTCGAGC[illegible]GCTTTTGGCTGTCAGGGCAGCTGTA     160
GGAGGCAG[illegible]AGAAGAGGGAG[illegible]GCCTTGGAGGAAGGGAAGGGGCCTGGTGCCGAGGA    240
CCTCTCCTGGCCAGGAGCTTCCTCCAGGACAAGACCTTCCACCCAACAAGGACTCCCCT
............................................................      80
................................iEEEEEEEEEEEEEEEEEEEEEEEEEEE     160
EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE     240
EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
```

- **W[illegible]t makes t[illegible] secor[illegible]ATG the TIS?**

Approach

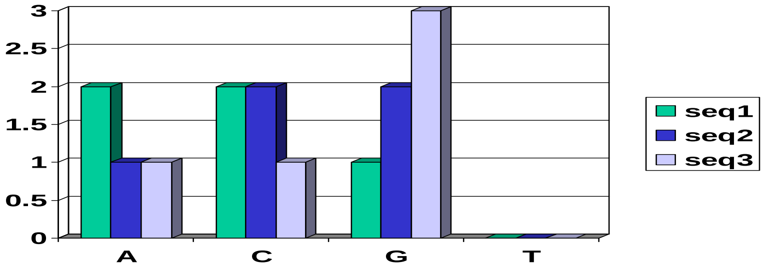
- **Training data gathering**
- **Signal generation**
  - – k-grams, distance, domain know-how, ...
- **Signal selection**
  - – Entropy, $\chi 2$, CFS, t-test, domain know-how...
- **Signal integration**
  - – SVM, ANN, PCL, CART, C4.5, kNN, ...

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, …
  - Window size vs. fixed position
  - Up-stream, downstream vs. any where in window
  - In-frame vs. any frame



Signal Generation: An Example

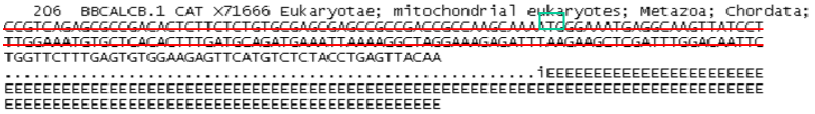
```
 299 HSU27655.1 CAT U27655 Homo sapiens
CGTGTGTGCAGC                                                 80
                                                            160
                                                   GAGGA    240
CCTCTCCTGGCCAGGAGCTTCCTCCAGGACAAGACCTTCCACCCAACAAGGACTCCCCT
```

Exercise: Find the in-frame downstream ATG

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

Feature Generation - Summary

Raw Data

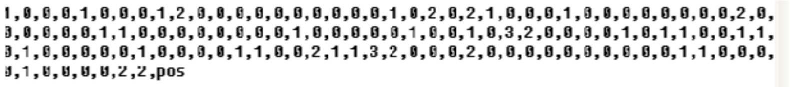


```
   206   BBCALCB.1 CAT X71666 Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
~~CCGTCAGAGCGCCGACACTCTTCTCTGTGCGAGCGAGCCGCCGACCGCCAAGCAAAATGGGAAATGAGGCAAGTTATCCT~~
~~TTGGAAATGTGCTCACACTTTGATGCAGATGAAATTAAAAGGCTAGGAAAGAGATTTAAGAAGCTCGATTTGGACAATTC~~
TGGTTCTTTGAGTGTGGAAGAGTTCATGTCTCTACCTGAGTTACAA
........................................................iEEEEEEEEEEEEEEEEEEEEEEE
EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE
```

An ATG segment – positive sample

```
> 206 +1_Index(56)
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCCGTCAGAGCGCCGACACTCTTCTCTGTGCGAGCGAG
CCGCCGACCGCCAAGCAAAATGGGAAATGAGGCAAGTTATCCTTTGGAAATGTGCTCACACTTTGATGCAGATGAAATTA
AAAGGCTAGGAAAGAGATTTAAGAAGCTCGATTTGGACAAT
```

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



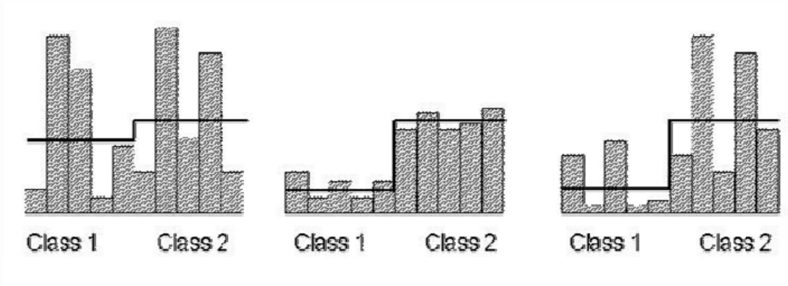
```
1,0,0,0,1,0,0,0,1,2,0,0,0,0,0,0,0,0,0,0,1,0,2,0,2,1,0,0,0,1,0,0,0,0,0,0,0,0,2,0,
0,0,0,0,0,1,1,0,0,0,0,0,0,0,0,1,0,0,0,0,0,1,0,0,1,0,3,2,0,0,0,0,1,0,1,1,0,0,1,1,
0,1,0,0,0,0,0,1,0,0,0,0,1,1,0,0,2,1,1,3,2,0,0,0,2,0,0,0,0,0,0,0,0,0,0,1,1,0,0,0,
0,1,0,0,0,0,2,2,pos
```

Too Many Features

- For each value of k, there are $4^k * 3 * 2$ 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 w/ low intra-class distance**
- **Choose a signal w/ high inter-class distance**



Signal Selection (e.g., t-statistics)

The t-stats of a signal is defined as

$$t = \frac{|\mu_1 - \mu_2|}{\sqrt{(\sigma_1^2/n_1) + (\sigma_2^2/n_2)}}$$

where σ_i^2 is the variance of that signal in class i, μ_i is the mean of that signal in class i, and n_i is the size of class i.

Signal Selection (e.g., MIT-correlation)

The MIT-correlation value of a signal is defined as

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

where σ_i is the standard deviation of that signal in class i and μ_i is the mean of that signal in class i.

Signal Selection (e.g., χ2)

The $\mathcal{X}^2$ value of a signal is defined as:

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

where m is the number of intervals, k the number of classes, A_{ij} the number of samples in the ith interval, jth class, R_i the number of samples in the ith interval, C_j the number of samples in the jth 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:**

	obs	exp	(obs – exp)^2/exp
HM	40	60*50/100=30	3.3
HW	20	60*50/100=30	3.3
LM	10	40*50/100=20	5.0
LW	30	40*50/100=20	5.0

χ2=16.6
P = 0.00004,
df = 1
So weight and
sex are not indep

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

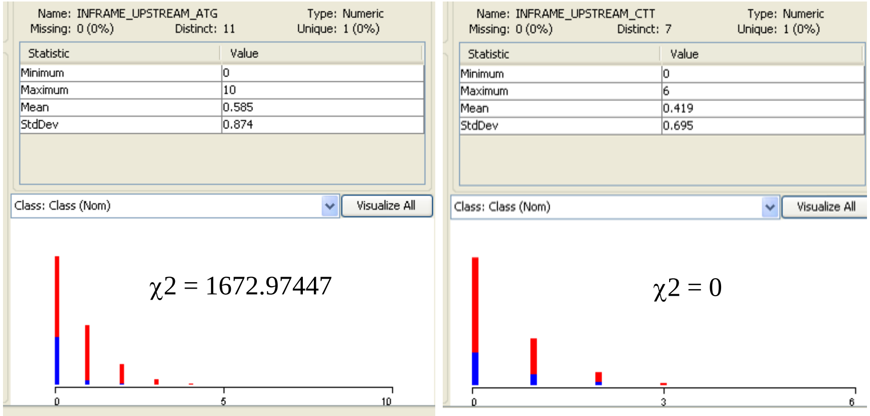
Signal Selection (e.g., CFS)

- **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

Exercise: What is the main challenge in implementing CFS?

Distributions of Two Example 3-Grams



Name: INFRAME_UPSTREAM_ATG Type: Numeric
Missing: 0 (0%) Distinct: 11 Unique: 1 (0%)

Statistic	Value
Minimum	0
Maximum	10
Mean	0.585
StdDev	0.874

Class: Class (Nom)

$\chi 2 = 1672.97447$

Name: INFRAME_UPSTREAM_CTT Type: Numeric
Missing: 0 (0%) Distinct: 7 Unique: 1 (0%)

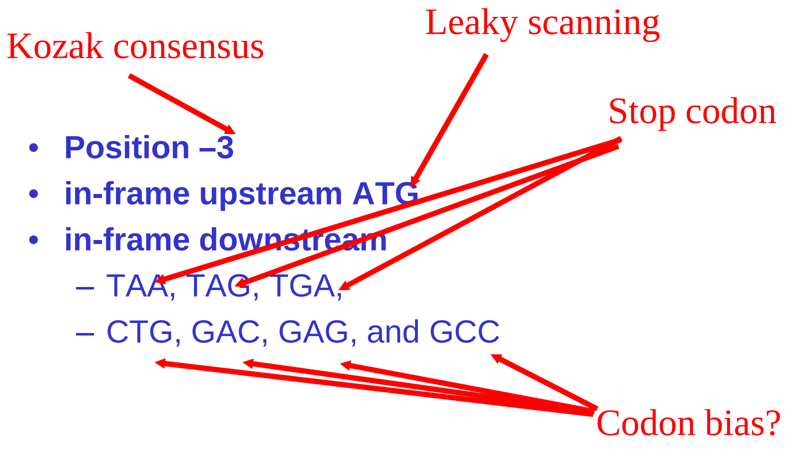
Statistic	Value
Minimum	0
Maximum	6
Mean	0.419
StdDev	0.695

Class: Class (Nom)

$\chi 2 = 0$

- **Which is the better one?**

Sample k-grams Selected by CFS for Recognizing TIS



Kozak consensus

Leaky scanning

Stop codon

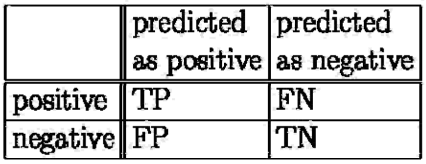
- **Position –3**
- **in-frame upstream ATG**
- **in-frame downstream**
  - TAA, TAG, TGA,
  - CTG, GAC, GAG, and GCC

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)



	predicted as positive	predicted as negative
positive	TP	FN
negative	FP	TN

Exercise:
What is TP/(TP+FP)?

	TP/(TP + FN)	TN/(TN + FP)	TP/(TP + FP)	Accuracy
Naïve Bayes	84.3%	86.1%	66.3%	85.7%
SVM	73.9%	93.2%	77.9%	88.5%
Neural Network	77.6%	93.2%	78.8%	89.4%
Decision Tree	74.0%	94.4%	81.1%	89.4%

Improvement by Voting

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

	TP/(TP + FN)	TN/(TN + FP)	TP/(TP + FP)	Accuracy
NB+SVM+NN	79.2%	92.1%	76.5%	88.9%
NB+SVM+Tree	78.8%	92.0%	76.2%	88.8%
NB+NN+Tree	77.6%	94.5%	82.1%	90.4%
SVM+NN+Tree	75.9%	94.3%	81.2%	89.8%
Best of 4	84.3%	94.4%	81.1%	89.4%
Worst of 4	73.9%	86.1%	66.3%	85.7%

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

	TP/(TP + FN)	TN/(TN + FP)	TP/(TP + FP)	Accuracy
NB	84.3%	86.1%	66.3%	85.7%
SVM	73.9%	93.2%	77.9%	88.5%
NB+Scanning	87.3%	96.1%	87.9%	93.9%
SVM+Scanning	88.5%	96.3%	88.6%	94.4%

Performance Comparisons

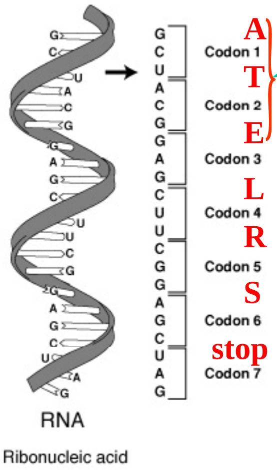
	TP/(TP + FN)	TN/(TN + FP)	TP/(TP + FP)	Accuracy
NB	84.3%	86.1%	66.3%	85.7%
Decision Tree	74.0%	94.4%	81.1%	89.4%
NB+NN+Tree	77.6%	94.5%	82.1%	90.4%
SVM+Scanning	88.5%	96.3%	88.6%	94.4%*
Pedersen&Nielsen	78%	87%	-	85%
Zien	69.9%	94.1%	-	88.1%
Hatzigeorgiou	-	-	-	94%*

* result not directly comparable

Technique Comparisons

- **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→protein



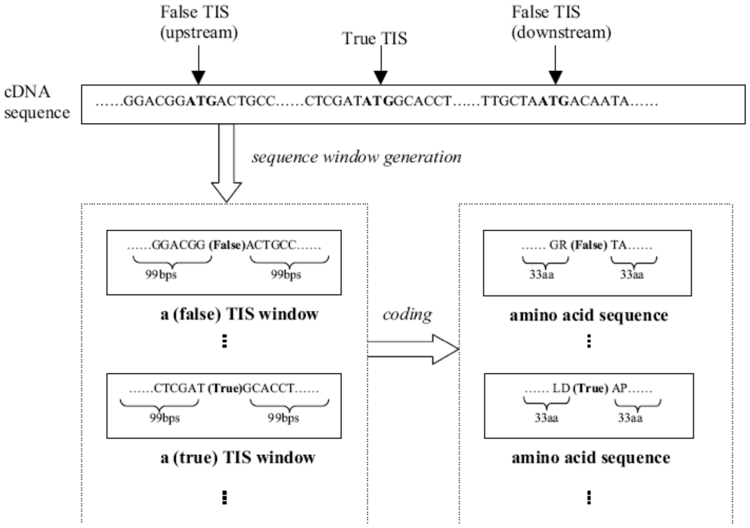
How about using k-grams from the translation?

First	U	C	A	G	Last
U	Phe F	Ser S	Tyr Y	Cys C	U
	Phe	Ser	Tyr	Cys	C
	Leu L	Ser	**Stop** (Ochre)	**Stop** (Umber)	A
	Leu	Ser	**Stop** (Amber)	Trp W	G
C	Leu	Pro P	His H	Arg R	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln Q	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile I	Thr T	Asn N	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys K	Arg	A
	Met M	Thr	Lys	Arg	G
G	Val V	Ala A	Asp D	Gly G	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu E	Gly	A
	Val	Ala	Glu	Gly	G

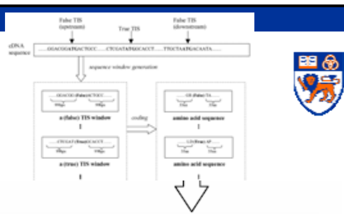
Exercise: List the first 10 amino acid in our example sequence

---

Amino-Acid Features

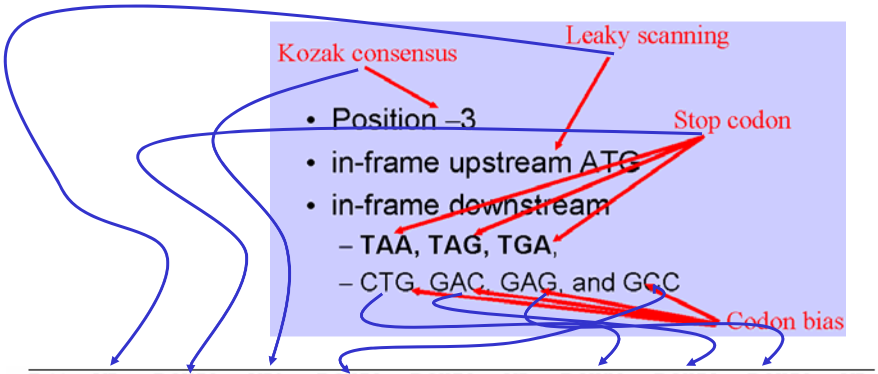


Amino-Acid Features



New feature space (total of 927 features + class label)			
42 1-gram amino acid patterns	882 2-gram amino acid patterns	3 bio-knowledge patterns	class label
UP-A, UP-R, ….,UP-N, DOWN-A, DOWN-R, …., DOWN-N (numeric type)	UP-AA, UP-AR, …., UP-NN, DOWN-AA, DOWN-AR , …, DOWN-NN (numeric type)	DOWN4-G UP3-AorG, UP-ATG (boolean type, Y or N)	True, False
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)



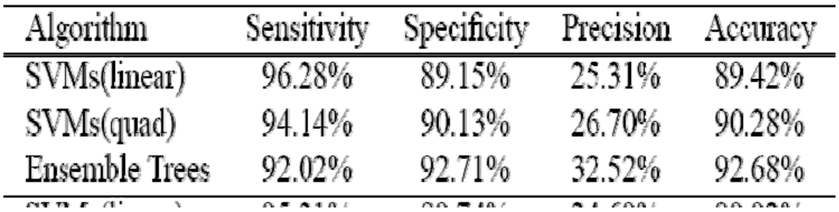
- Position –3
- in-frame upstream ATG
- in-frame downstream
  - **TAA, TAG, TGA,**
  - CTG, GAC, GAG, and GCC

Fold	UP-ATG	DOWN-STOP	UP3-AorG	DOWN-A	DOWN-V	UP-A	DOWN-L	DOWN-D	DOWN-E	UP-G
1	1	2	4	3	6	5	8	9	7	10
2	1	2	3	4	5	6	7	8	9	10
3	1	2	3	4	5	6	8	9	7	10

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)

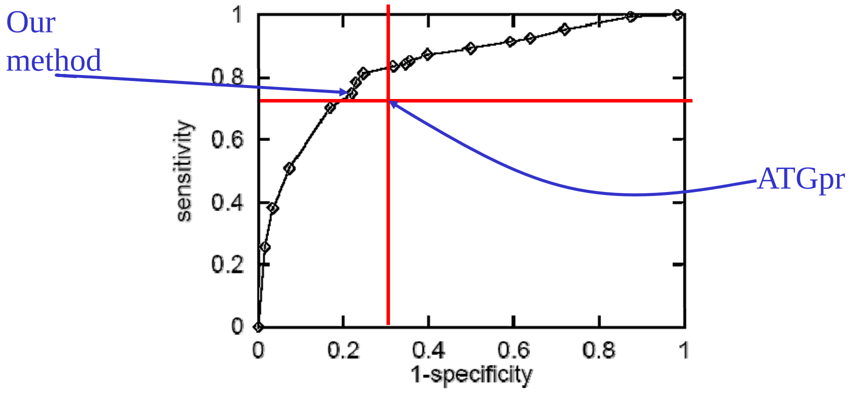
Validation Results (on Hatzigeorgiou's)



Algorithm	Sensitivity	Specificity	Precision	Accuracy
SVMs(linear)	96.28%	89.15%	25.31%	89.42%
SVMs(quad)	94.14%	90.13%	26.70%	90.28%
Ensemble Trees	92.02%	92.71%	32.52%	92.68%

- – Using top 100 features selected by entropy and trained on Pedersen & Nielsen's dataset

Validation Results (on Chr X and Chr 21)



Our method

ATGpr

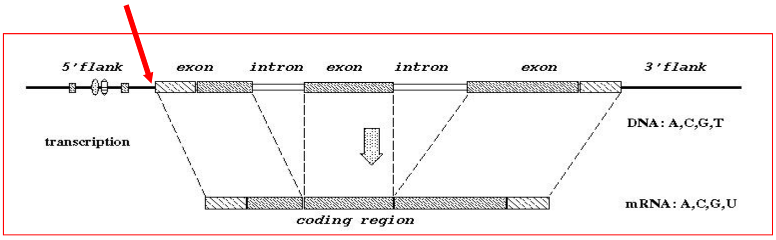
- **Using top 100 features selected by entropy and trained on Pedersen & Nielsen’s**

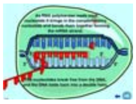
Recognition of Transcription Start Sites

An introduction to the World’s best TSS recognition system:

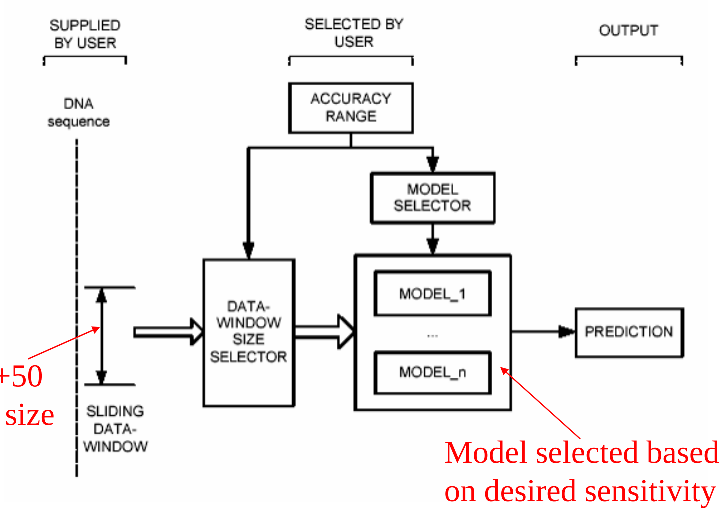
A heavy tuning approach

Transcription Start Site





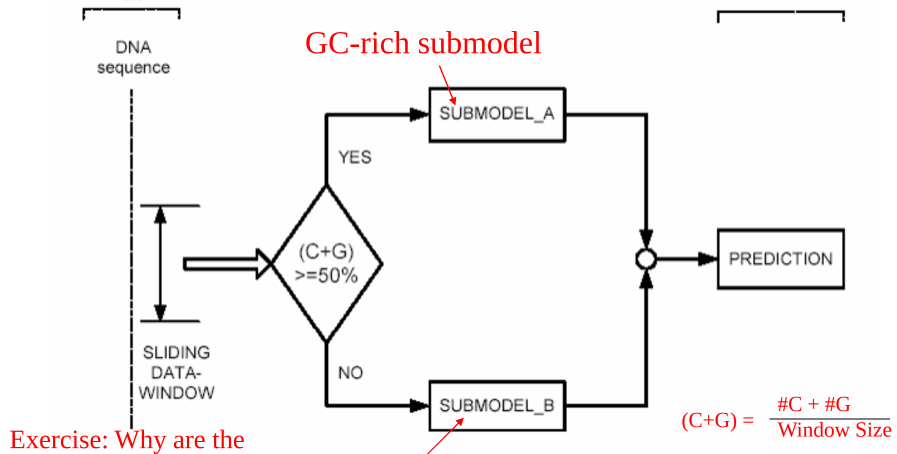
Structure of Dragon Promoter Finder



-200 to +50 window size

Model selected based on desired sensitivity

Each model has two submodels based on GC content



SUPPLIED BY USER

OUTPUT

GC-rich submodel

DNA sequence

SUBMODEL_A

YES

(C+G) >=50%

PREDICTION

SLIDING DATA-WINDOW

NO

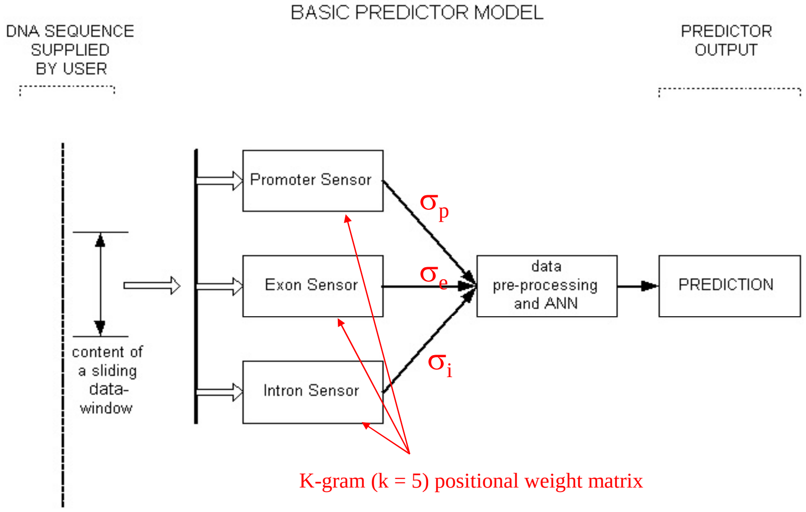
SUBMODEL_B

$(C+G) = \frac{\#C + \#G}{\text{Window Size}}$

Exercise: Why are the submodels based on GC content?

GC-poor submodel

Data Analysis Within Submodel



BASIC PREDICTOR MODEL

DNA SEQUENCE SUPPLIED BY USER

PREDICTOR OUTPUT

Promoter Sensor

σ_p

Exon Sensor

σ_e

data pre-processing and ANN

PREDICTION

content of a sliding data-window

Intron Sensor

σ_i

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**

Pentamer at ith position in input

Window size

$$\sigma = \frac{\left(\sum_{i=1}^{L-4} p_j^i \otimes f_{j,i}\right)}{\left(\sum_{i=1}^{L-4} \max_j f_{j,i}\right)}, \qquad p_j^i \otimes f_{j,i} = \begin{cases} f_{j,i}, \text{ if } p_i = p_j^i \\ 0, \text{ if } p_i \neq p_j^i \end{cases},$$

Frequency of jth pentamer at ith position in training window

jth pentamer at ith 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**

1-mer	**pos1**	**pos2**	**pos3**	**pos4**	**pos5**	**pos6**	**pos7**	**pos8**	**pos9**	**pos10**
A	**3/3**	**0/3**	**0/3**							
C	**0/3**	**1/3**	**1/3**		Exercise: Fill in the rest of the table					
G	**0/3**	**2/3**	**0/3**							
T	**0/3**	**0/3**	**2/3**							

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**

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

2-mer	pos1	pos2	pos3	pos4	pos5	pos6	pos7	pos8	pos9
AA	**0/3**	**0/3**	**0/3**						
AC	**1/3**	**0/3**	**0/3**		Exercise: Fill in the rest of the table				
…	**…**	**…**	**…**						
TT	**0/3**	**0/3**	**1/3**				**1/3**		

Data Preprocessing & ANN

Tuning parameters

$s_E = sat(\sigma_p - \sigma_e, a_e, b_e)$,

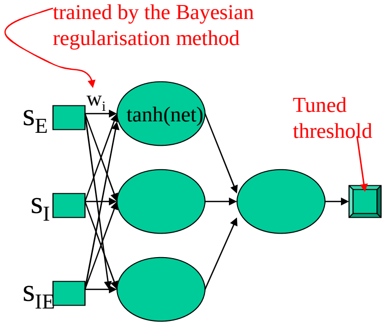
$s_I = sat(\sigma_p - \sigma_i, a_i, b_i)$,

$s_{EI} = sat(\sigma_e - \sigma_i, a_{ei}, b_{ei})$,

where the function *sat* is defined by

$$sat(x,a,b) = \begin{cases} a, & if \quad x > a \\ x, & if \quad b \le x \le a. \\ b, & if \quad b > x \end{cases}$$

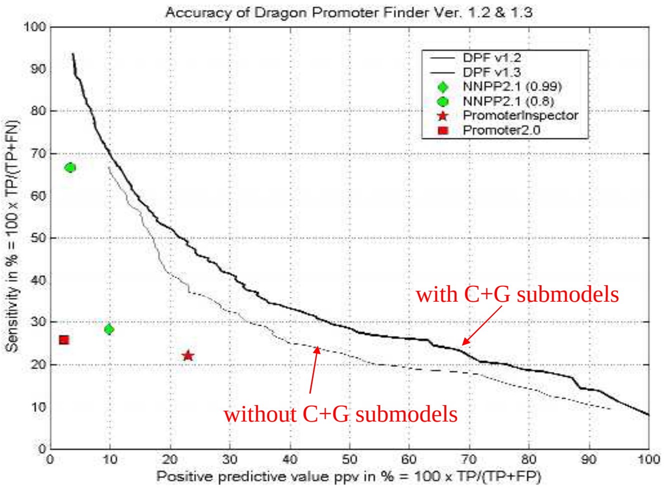
Simple feedforward ANN trained by the Bayesian regularisation method



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

$$net = \Sigma\, s_i * w_i$$

Accuracy Comparisons



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**
- **cummulative length of more than 1.15Mbp**
- **Taken from GENESCAN, GeneId, Genie, etc.**

Recognition of Poly-A Signal Sites

A twist to the "feature generation, feature selection, feature integration" approach

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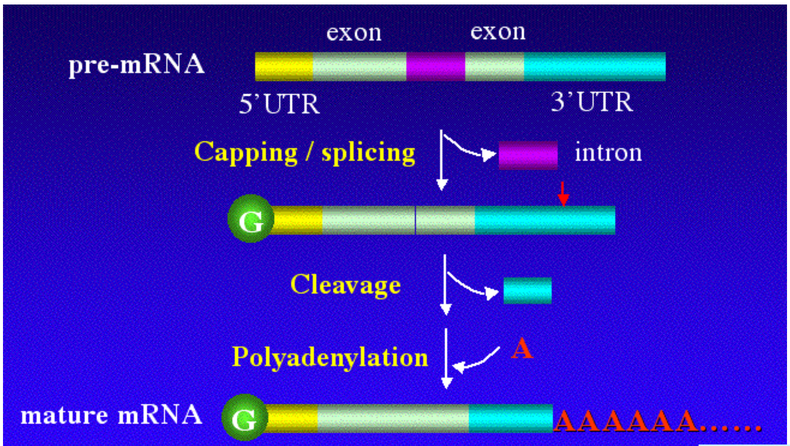
Eukaryotic Pre-mRNA Processing



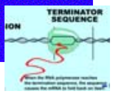


Image credit: www.polya.org

Poly-A Signals in Human (Gautheret et al., 2000)

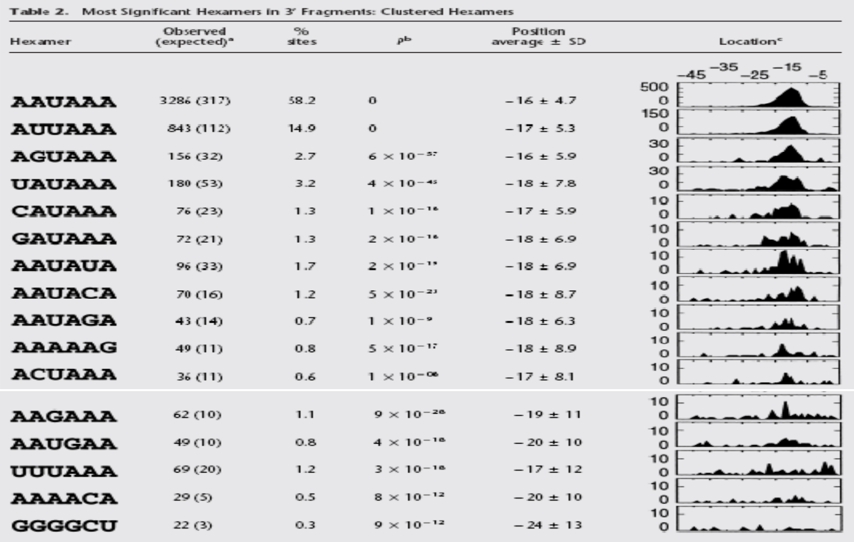
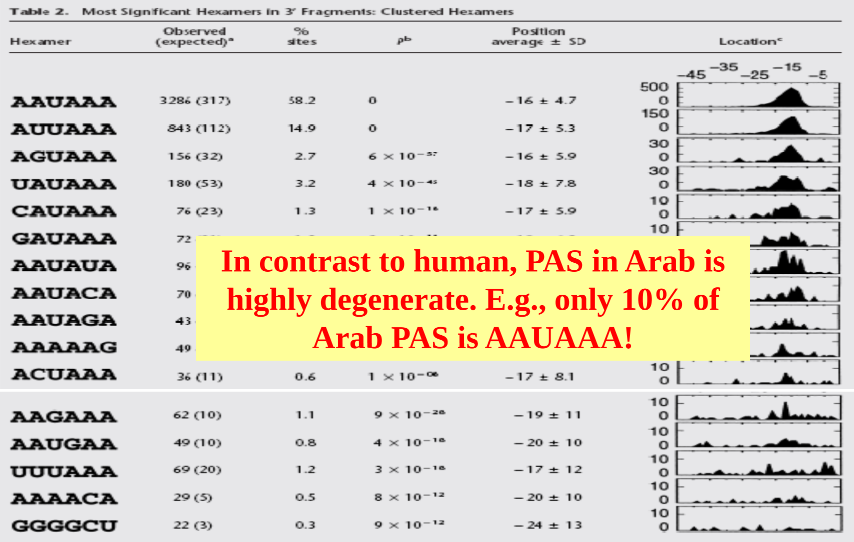


Table 2. Most Significant Hexamers in 3′ Fragments: Clustered Hexamers

Hexamer	Observed (expected)[a]	% sites	p^b	Position average ± SD	Location[c]
AAUAAA	3286 (317)	58.2	0	−16 ± 4.7	500 0
AUUAAA	843 (112)	14.9	0	−17 ± 5.3	150 0
AGUAAA	156 (32)	2.7	6×10^{-57}	−16 ± 5.9	30 0
UAUAAA	180 (53)	3.2	4×10^{-45}	−18 ± 7.8	30 0
CAUAAA	76 (23)	1.3	1×10^{-16}	−17 ± 5.9	10 0
GAUAAA	72 (21)	1.3	2×10^{-16}	−18 ± 6.9	10 0
AAUAUA	96 (33)	1.7	2×10^{-15}	−18 ± 6.9	10 0
AAUACA	70 (16)	1.2	5×10^{-23}	−18 ± 8.7	10 0
AAUAGA	43 (14)	0.7	1×10^{-9}	−18 ± 6.3	10 0
AAAAAG	49 (11)	0.8	5×10^{-17}	−18 ± 8.9	10 0
ACUAAA	36 (11)	0.6	1×10^{-08}	−17 ± 8.1	10 0
AAGAAA	62 (10)	1.1	9×10^{-28}	−19 ± 11	10 0
AAUGAA	49 (10)	0.8	4×10^{-18}	−20 ± 10	10 0
UUUAAA	69 (20)	1.2	3×10^{-18}	−17 ± 12	10 0
AAAACA	29 (5)	0.5	8×10^{-12}	−20 ± 10	10 0
GGGGCU	22 (3)	0.3	9×10^{-12}	−24 ± 13	10 0

Poly-A Signals in Arabidopsis



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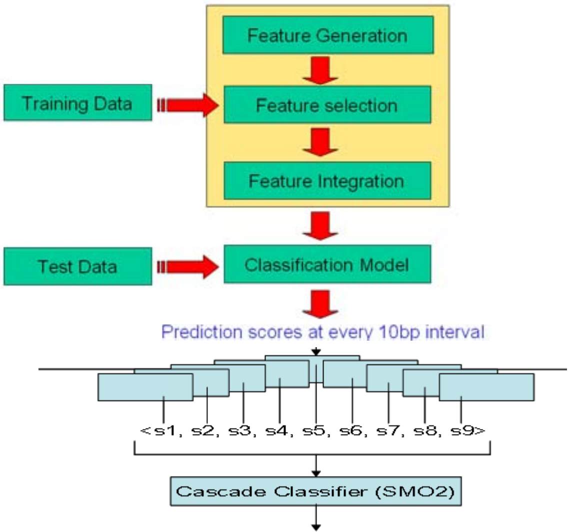
AAAACA	29 (5)	0.5	8×10^{-12}	−20 ± 10	10 0
GGGGCU	22 (3)	0.3	9×10^{-12}	−24 ± 13	10 0

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)



Feature Generation

Training Data

Feature selection

Feature Integration

Test Data

Classification Model

Prediction scores at every 10bp interval

<s1, s2, s3, s4, s5, s6, s7, s8, s9>

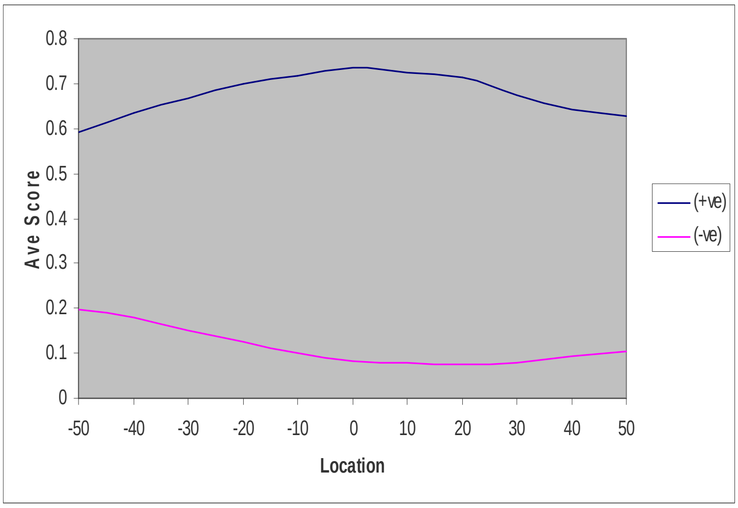
Cascade Classifier (SMO2)

(+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



Validation Results

SN_0	**SMO 1**		**SMO 2**		**PASS 1.0**	
Control Sequences	SN & SP	Threshold	SN & SP	Threshold	SN & SP	Threshold
CDS	90%	0.26	94%	0.24	95%	3.7
5'UTR	79%	0.42	85%	0.49	78%	5.5
Intron	64%	0.59	71%	0.67	63%	6.3

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

SN_10	**SMO 1**		**SMO 2**		**PASS 1.0**	
Control Sequences	SN & SP	Threshold	SN & SP	Threshold	SN & SP	Threshold
CDS	94%	0.36	96%	0.31	96%	4
5'UTR	86%	0.53	89%	0.6	81%	5.7
Intron	73%	0.68	77%	0.77	67%	6.6

Table 3. Equal-error-rate points of SMO1, SMO2, and PASS 1.0 for SN_30.

SN_30	**SMO 1**		**SMO 2**		**PASS 1.0**	
Control Sequences	SN & SP	Threshold	SN & SP	Threshold	SN & SP	Threshold
CDS	97%	0.44	97%	0.37	97%	4.3
5'UTR	90%	0.62	92%	0.67	84%	6.2
Intron	79%	0.75	83%	0.81	72%	6.8

Concluding Remarks…

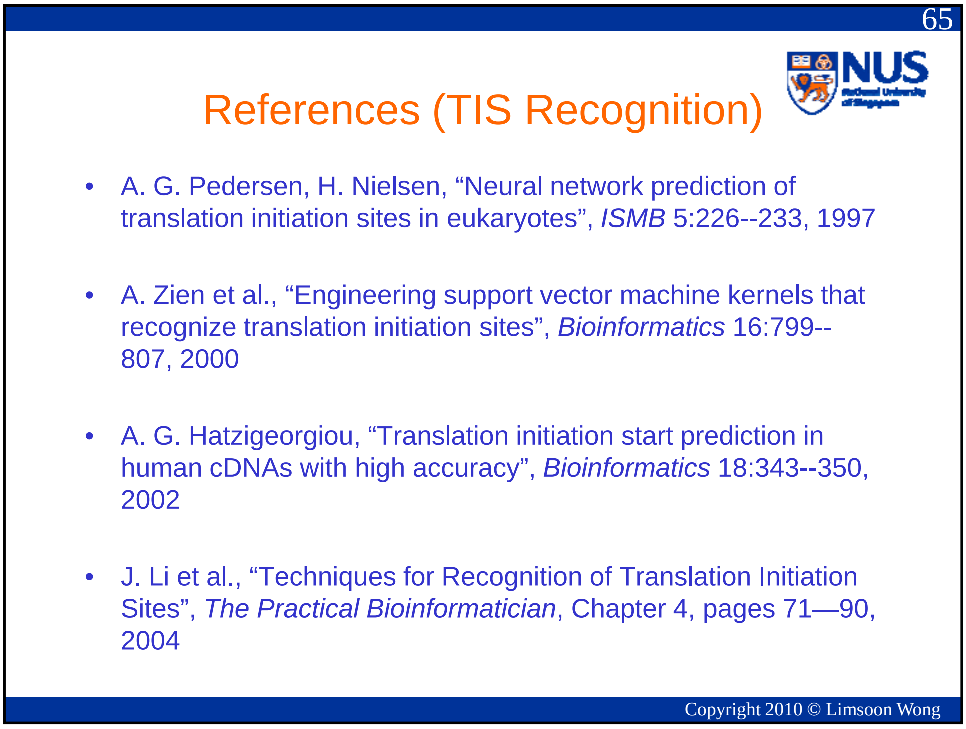
What have we learned?

- **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**



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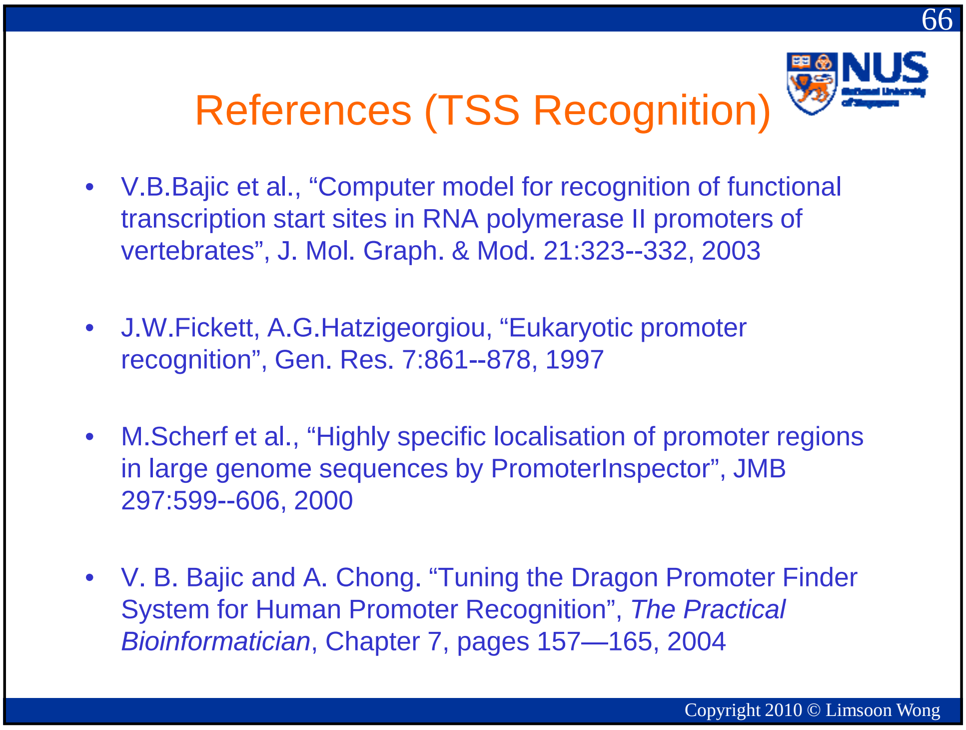

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