CSE 527
Computational Biology
Autumn 2005

Lectures ~15-16
Gene Prediction

Some References

- A great online bib
  - http://www.nslij-genetics.org/gene/
- A good intro survey
  - JM Claverie (1997) "Computational methods for the identification of genes in vertebrate genomic sequences" Human Molecular Genetics, 6(10)(review issue): 1735-1744.
- A gene finding bake-off
  - M Burset, R Guigo (1996), "Evaluation of gene structure prediction programs", Genomics, 34(3): 353-367.

Motivation

- Sequence data flooding into Genbank
- What does it mean?

  protein genes, RNA genes, mitochondria, chloroplast, regulation, replication, structure, repeats, transposons, unknown stuff, …

Protein Coding Nuclear DNA

- Focus of next 2 lectures
- Goal: Automated annotation of new sequence data
- State of the Art:
  - predictions ~ 60% similar to real proteins
  - ~80% if database similarity used
  - lab verification still needed, still expensive

Biological Basics

- Central Dogma:

  $$\text{DNA} \xrightarrow{\text{transcription}} \text{RNA} \xrightarrow{\text{translation}} \text{Protein}$$

- Codons: 3 bases code one amino acid
  - Start codon
  - Stop codons
  - 3’, 5’ Untranslated Regions (UTR’s)

The Genetic Code

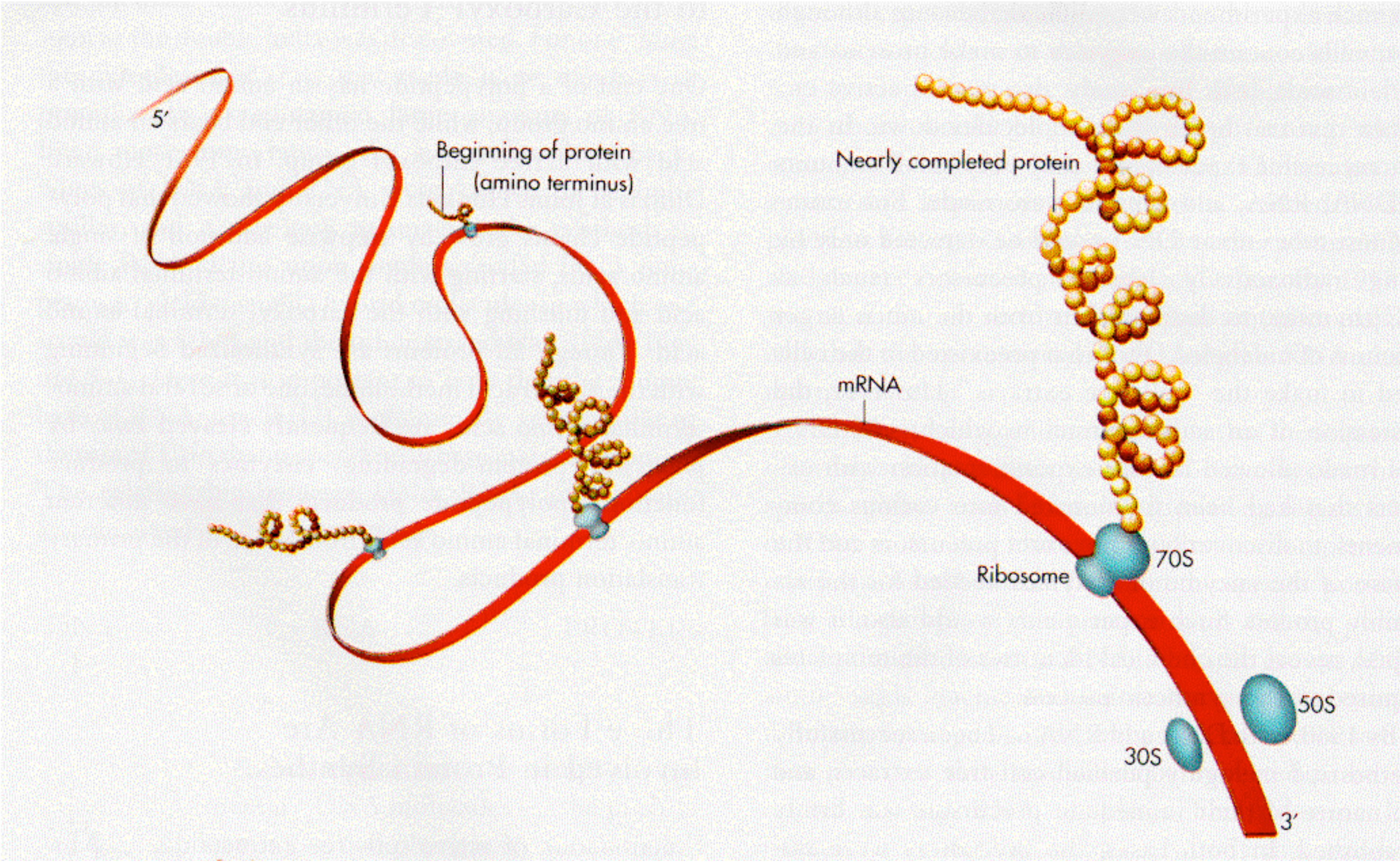
(a) RNA Codons for the Twenty Amino Acids

First base	Second base: U	C	A	G	Third base
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met (start)	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Amino-acid abbreviations

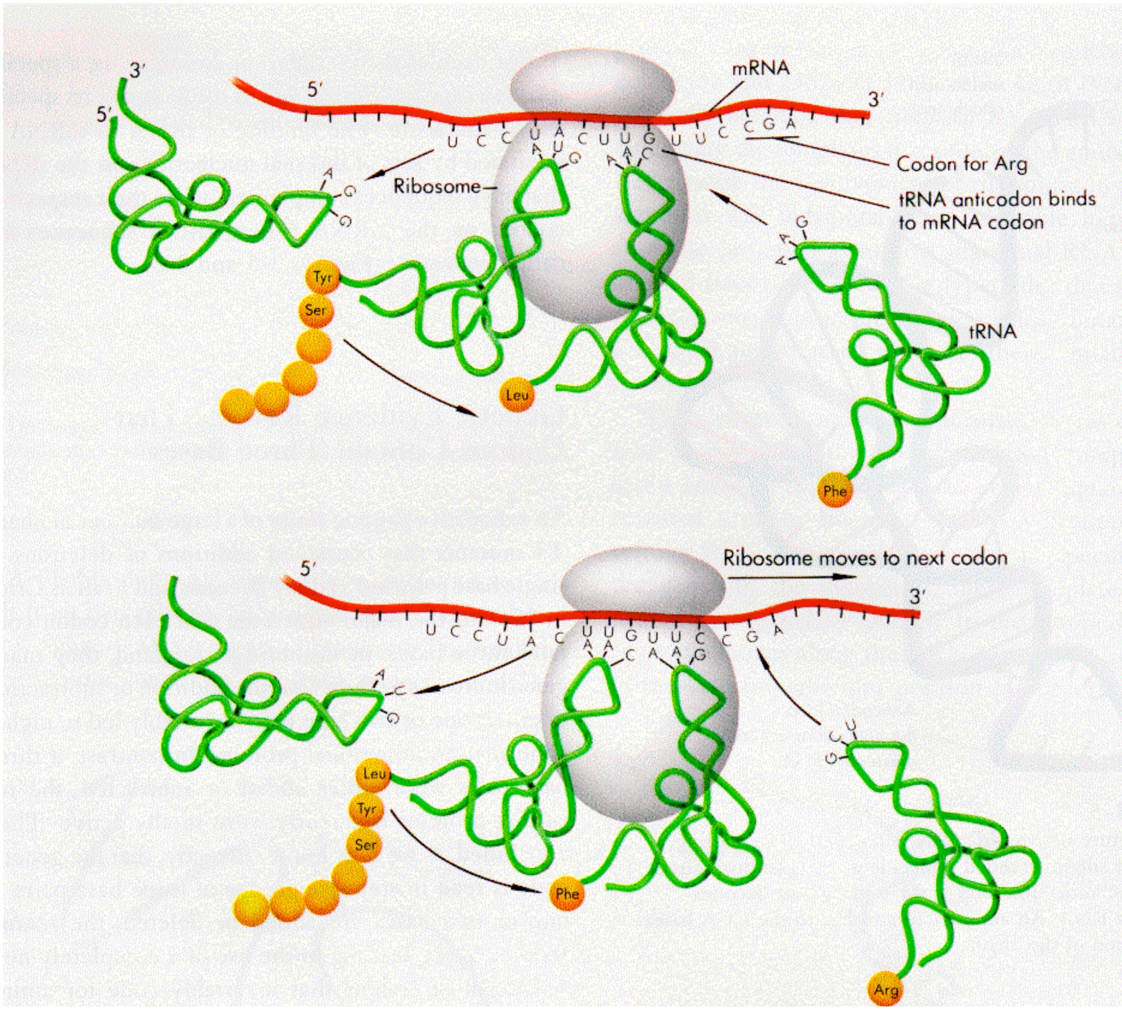
Ala = Alanine
Arg = Arginine
Asp = Aspartic acid
Asn = Asparagine
Cys = Cysteine
Glu = Glutamic acid
Gln = Glutamine
Gly = Glycine
His = Histidine
Ile = Isoleucine
Leu = Leucine
Lys = Lysine
Met = Methionine
Phe = Phenylalanine
Pro = Proline
Ser = Serine
Thr = Threonine
Trp = Tryptophan
Tyr = Tyrosine
Val = Valine

Translation: mRNA → Protein



Watson, Gilman, Witkowski, & Zoller, 1992

Ribosomes



Watson, Gilman, Witkowski, & Zoller, 1992

Idea #1: Find Long ORF's

- Reading frame: which of the 3 possible sequences of triples does the ribosome read?
- Open Reading Frame: No stop codons
- In random DNA
  - average ORF = 64/3 = 21 triplets
  - 300bp ORF once per 36kbp per strand
- But average protein ~ 1000bp

Idea #2: Codon Frequency

- In random DNA

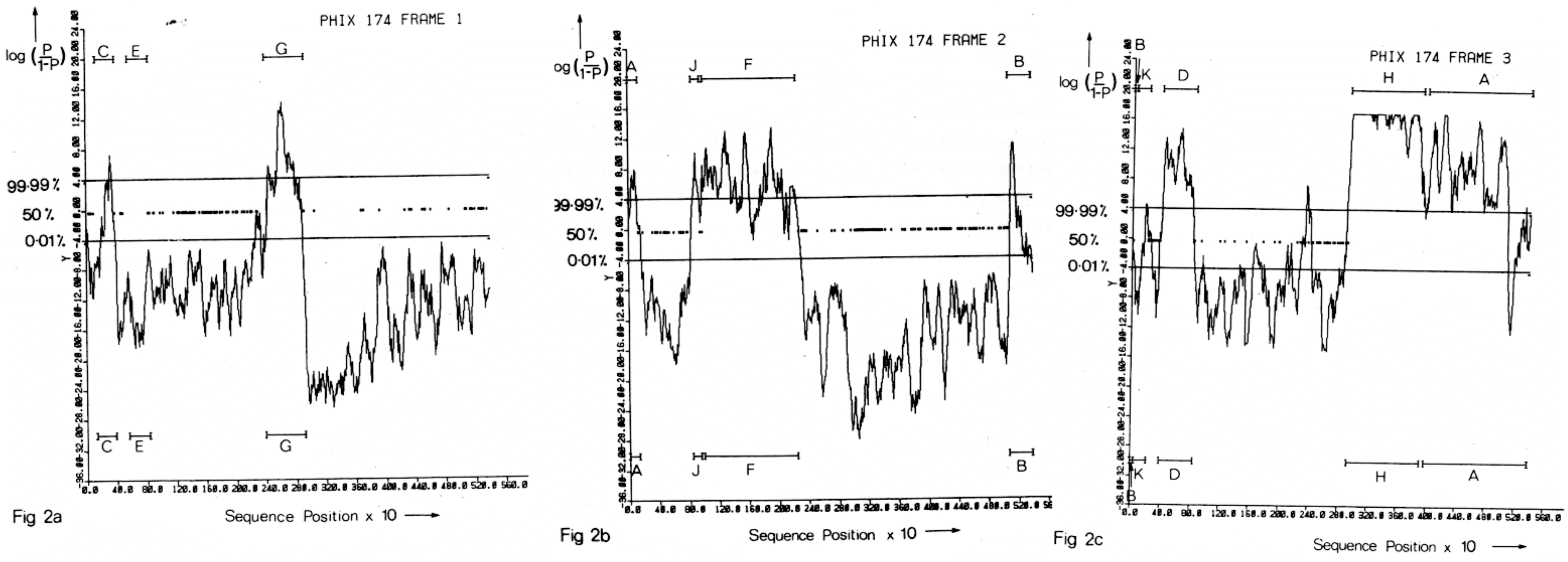
  Leucine : Alanine : Tryptophan = 6 : 4 : 1
- But in real protein, ratios ~ 6.9 : 6.5 : 1
- So, coding DNA is not random
- Even more: synonym usage is biased (in a species dependant way)

  examples known with 90% AT 3rd base

Recognizing Codon Bias

- Assume
  - Codon usage i.i.d.; abc with freq. f(abc)
  - $a_1a_2a_3a_4 \ldots a_{3n+2}$ is coding, unknown frame
- Calculate
  - $p_1 = f(a_1a_2a_3)f(a_4a_5a_6) \ldots f(a_{3n-2}a_{3n-1}a_{3n})$
  - $p_2 = f(a_2a_3a_4)f(a_5a_6a_7) \ldots f(a_{3n-1}a_{3n}\, a_{3n+1})$
  - $p_3 = f(a_3a_4a_5)f(a_6a_7a_8) \ldots f(a_{3n}\, a_{3n+1}a_{3n+2})$
  - $P_i = p_i / (p_1+p_1+p_3)$
- More generally: k-th order Markov model
  - k=5 or 6 is typical

Codon Usage in Φx174



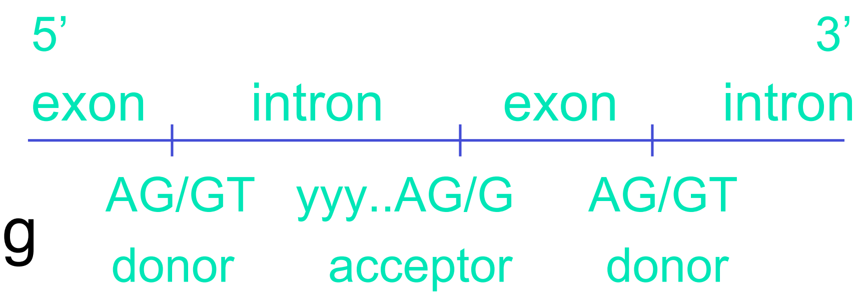
Staden & McLachlan, NAR 10, 1 1982, 141-156

Promoters, etc.

- In prokaryotes, most DNA coding
  E.g. ~ 70% in *H. influenzae*
- Long ORFs + codon stats do well
- But obviously won't be perfect
  - short genes
  - 5' & 3' UTR's
- Can improve by modeling promoters & other signals
  - e.g. via WMM or higher-order Markov models

Eukaryotes

- As in prokaryotes (but maybe more variable)
  - promoters
  - start/stop transcription
  - start/stop translation
- New Features:
  - polyA site/tail
  - introns, exons, splicing
  - branch point signal
  - alternative splicing



Characteristics of human genes
(Nature, 2/2001, Table 21)

	Median	Mean	Sample (size)
Internal exon	122 bp	145 bp	RefSeq alignments to draft genome sequence, with confirmed intron boundaries (43,317 exons)
Exon number	7	8.8	RefSeq alignments to finished sequence (3,501 genes)
Introns	1,023 bp	3,365 bp	RefSeq alignments to finished sequence (27,238 introns)
3' UTR	400 bp	770 bp	Confirmed by mRNA or EST on chromo 22 (689)
5' UTR	240 bp	300 bp	Confirmed by mRNA or EST on chromo 22 (463)
Coding seq	1,100 bp	1340bp	Selected RefSeq entries (1,804)*
(CDS)	367 aa	447 aa	
Genomic extent	14 kb	27 kb	Selected RefSeq entries (1,804)*

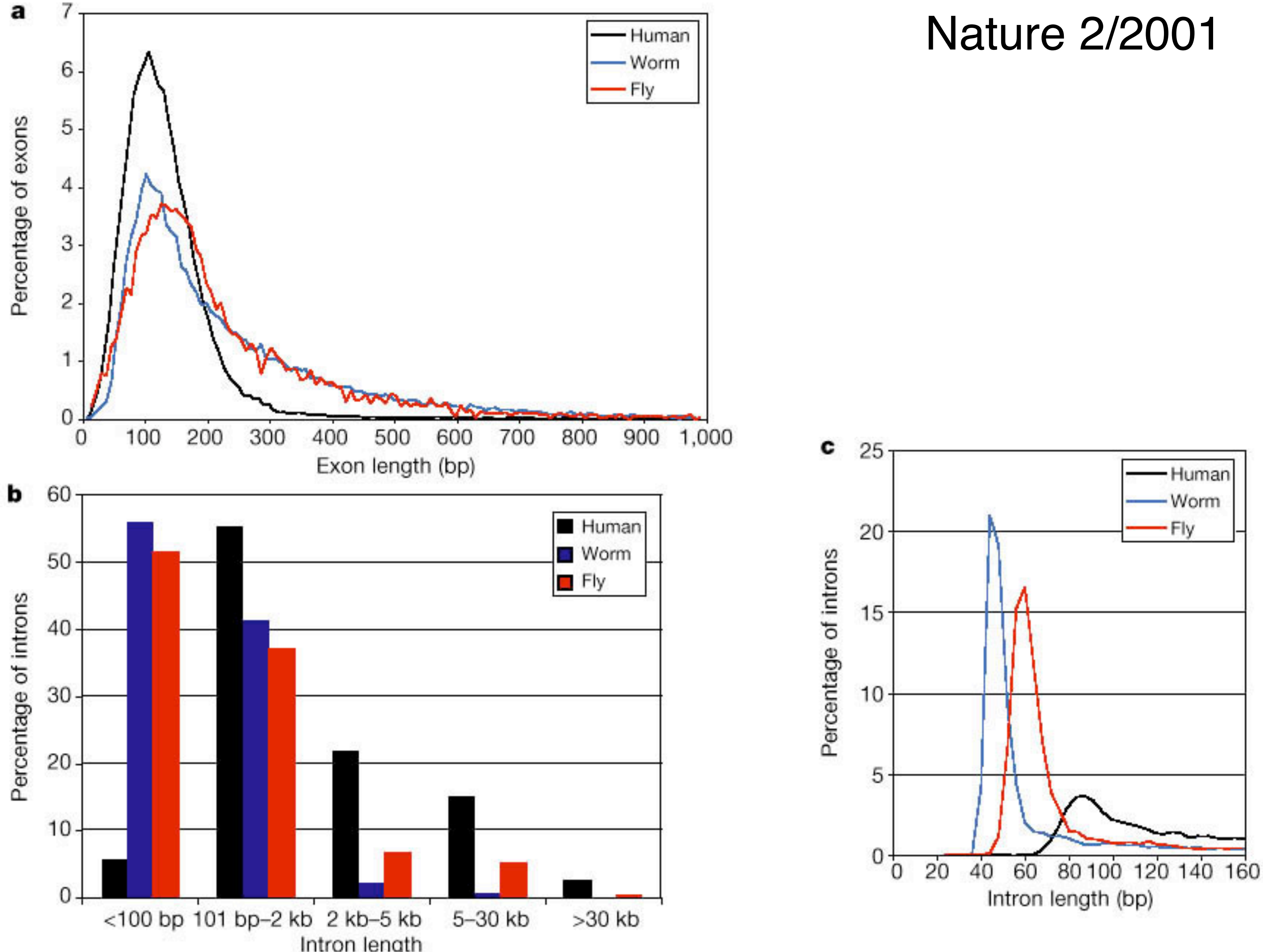
* 1,804 selected RefSeq entries were those with full-length unambiguous alignment to finished sequence

Big Genes

- Many genes are over 100 kb long,
- Max known: dystrophin gene (DMD), 2.4 Mb.
- The variation in the size distribution of coding sequences and exons is less extreme, although there are remarkable outliers.
    - The titin gene has the longest currently known coding sequence at 80,780 bp; it also has the largest number of exons (178) and longest single exon (17,106 bp).

RNApol rate: 2.5 kb/min

Nature 2/2001



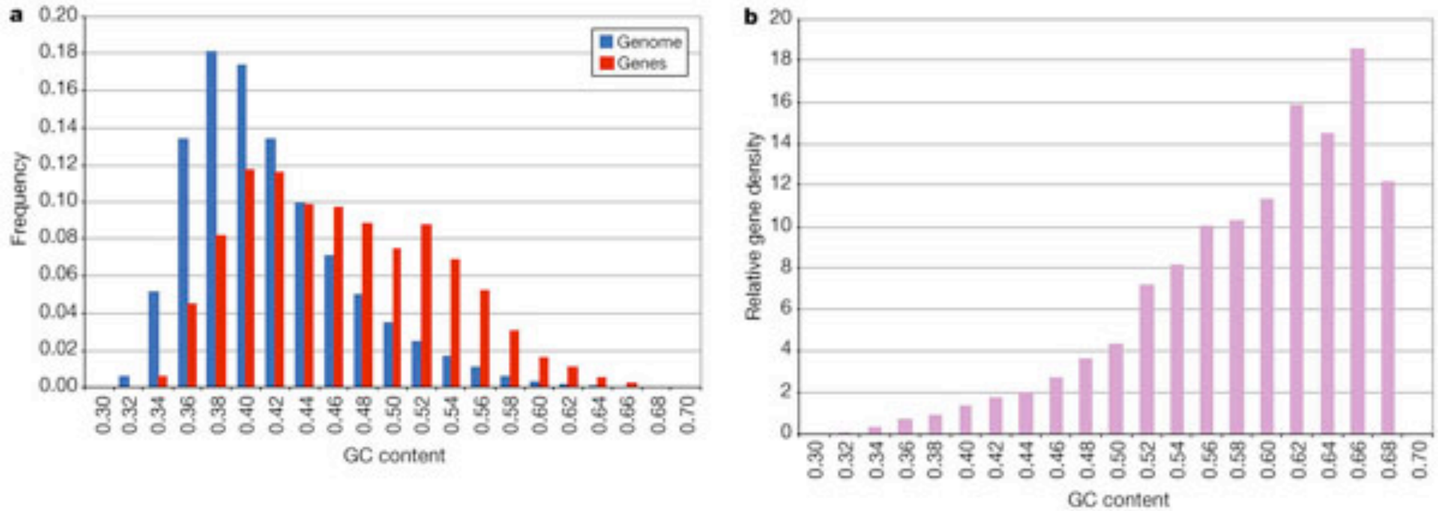
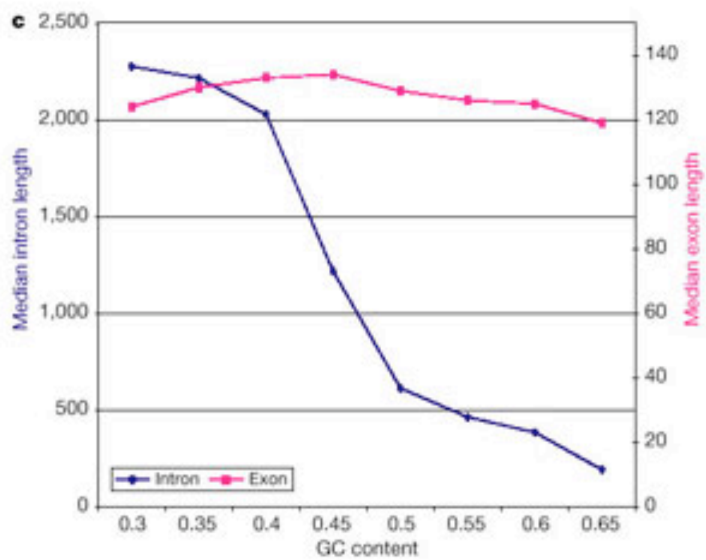


Figure 36 GC content. a, Distribution of GC content in genes and in the genome. For 9,315 known genes mapped to the draft genome sequence, the local GC content was calculated in a window covering either the whole alignment or 20,000 bp centred around the midpoint of the alignment, whichever was larger. Ns in the sequence were not counted. GC content for the genome was calculated for adjacent nonoverlapping 20,000-bp windows across the sequence. Both the gene and genome distributions have been normalized to sum to one.



b, Gene density as a function of GC content, obtained by taking the ratio of the data in a. Values are less accurate at higher GC levels because the denominator is small. c, Dependence of mean exon and intron lengths on GC content. For exons and introns, the local GC content was derived from alignments to finished sequence only, and were calculated from windows covering the feature or 10,000 bp centred on the feature, whichever was larger.

A Case Study -- Genscan

- C Burge, S Karlin (1997), "Prediction of complete gene structures in human genomic DNA", Journal of Molecular Biology , 268: 78-94.

Training Data

- 238 multi-exon genes
- 142 single-exon genes
- total of 1492 exons
- total of 1254 introns
- total of 2.5 Mb

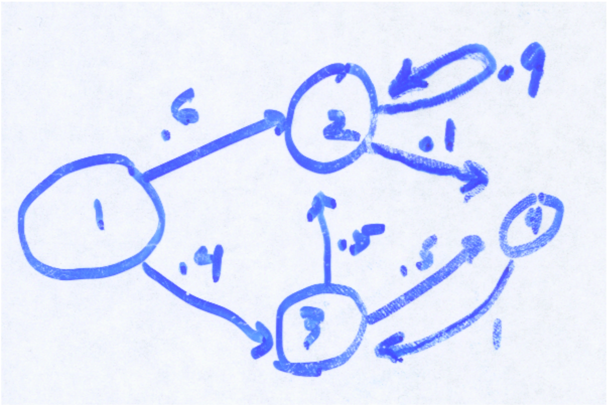
- NO alternate splicing, none > 30kb, ...

Performance Comparison

	Accuracy						
	per nuc.		per exon				
Program	**Sn**	**Sp**	**Sn**	**Sp**	**Avg.**	**ME**	**WE**
GENSCAN	0.93	0.93	0.78	0.81	0.80	0.09	0.05
FGENEH	0.77	0.88	0.61	0.64	0.64	0.15	0.12
GeneID	0.63	0.81	0.44	0.46	0.45	0.28	0.24
Genie	0.76	0.77	0.55	0.48	0.51	0.17	0.33
GenLang	0.72	0.79	0.51	0.52	0.52	0.21	0.22
GeneParser2	0.66	0.79	0.35	0.40	0.37	0.34	0.17
GRAIL2	0.72	0.87	0.36	0.43	0.40	0.25	0.11
SORFIND	0.71	0.85	0.42	0.47	0.45	0.24	0.14
Xpound	0.61	0.87	0.15	0.18	0.17	0.33	0.13
GeneID‡	0.91	0.91	0.73	0.70	0.71	0.07	0.13
GeneParser3	0.86	0.91	0.56	0.58	0.57	0.14	0.09

After Burge&Karlin, Table 1. Sensitivity, Sn = TP/AP; Specificity, Sp = TP/PP

Generalized Hidden Markov Models



- π: Initial state distribution
- a_{ij}: Transition probabilities
- One submodel per state
- Outputs are *strings* gen'ed by submodel
- Given length L
  - Pick start state q_1 ($\sim\pi$)
  - While $\sum d_i < L$
    - Pick string s_i of length $d_i = |s_i|$ ~ submodel for q_i
    - Pick next state q_{i+1} ($\sim a_{ij}$)
  - Output $s_1 s_2 \ldots$

"Parse" ϕ of $S = s_1 s_2 \cdots s_L$

is $d_1 d_2 \cdots d_k$ s.t. $\sum d_i = L$

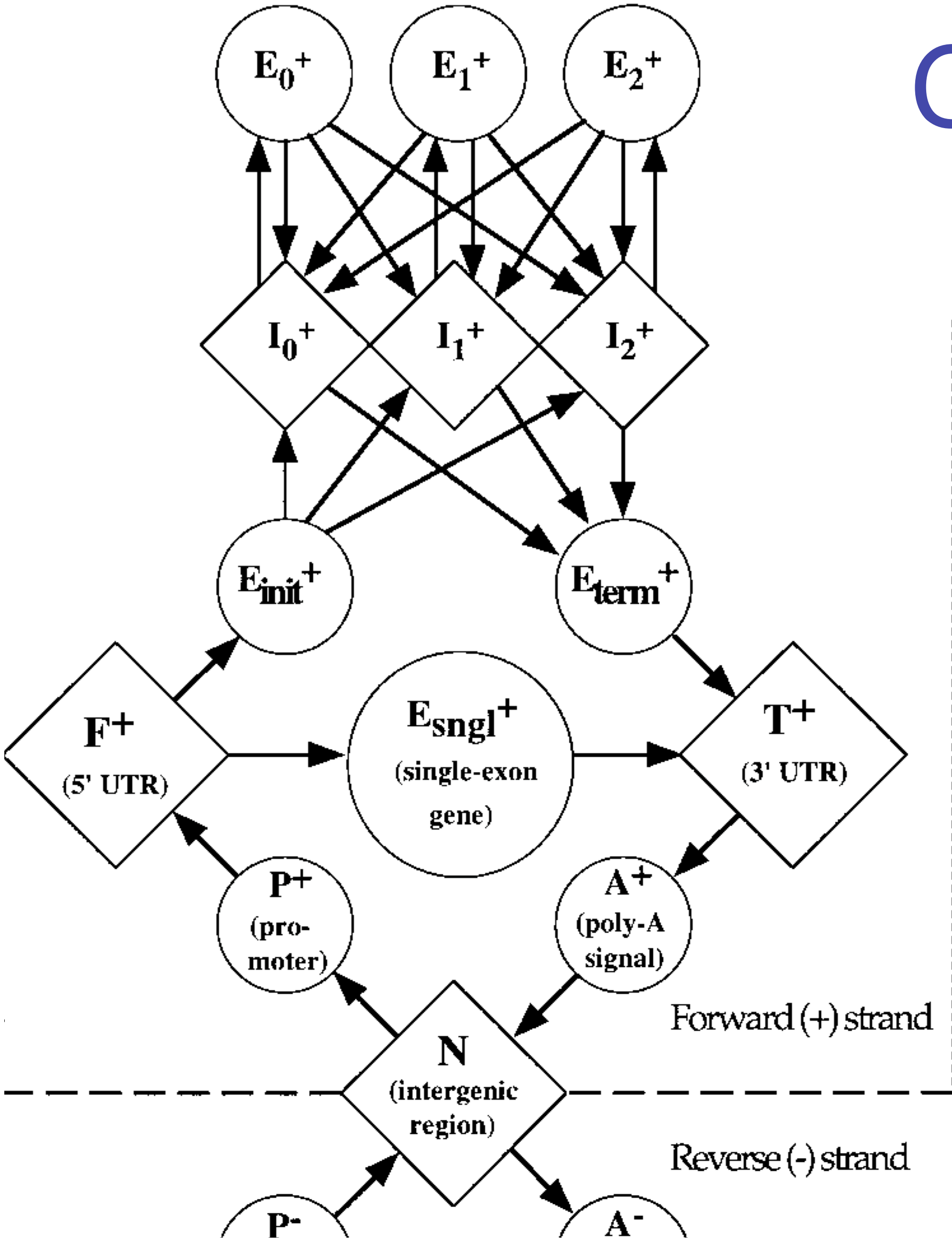
& $q_1 q_2 \cdots q_k$

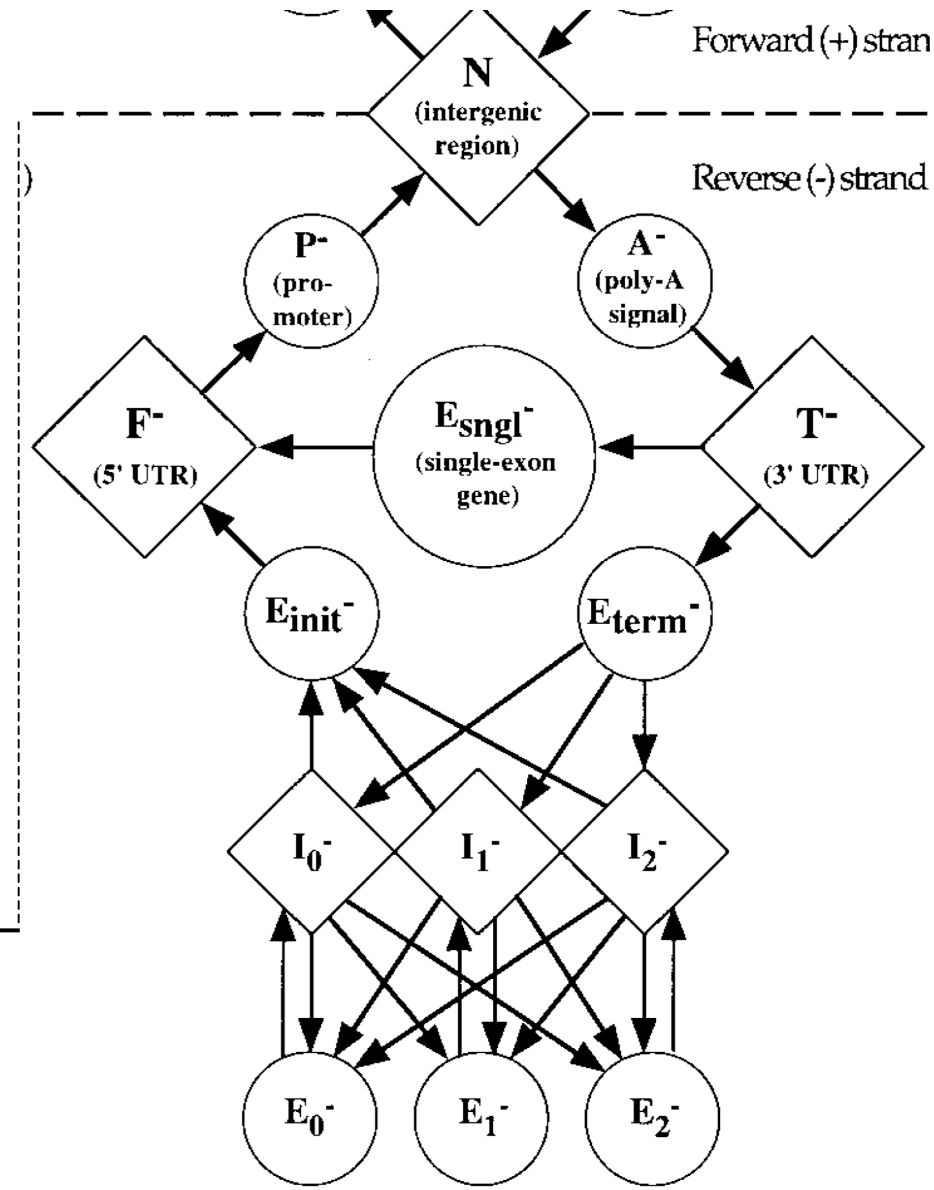
$$\Pr(\phi \mid S) = \frac{\Pr(\phi \wedge S)}{\Pr(S)}$$

$\vdots$

E.g. Use something like forward/backward to calc. Prob that positions $i \ldots j$ are an exon of phase k in S.

GHMM Structure





Length Distributions

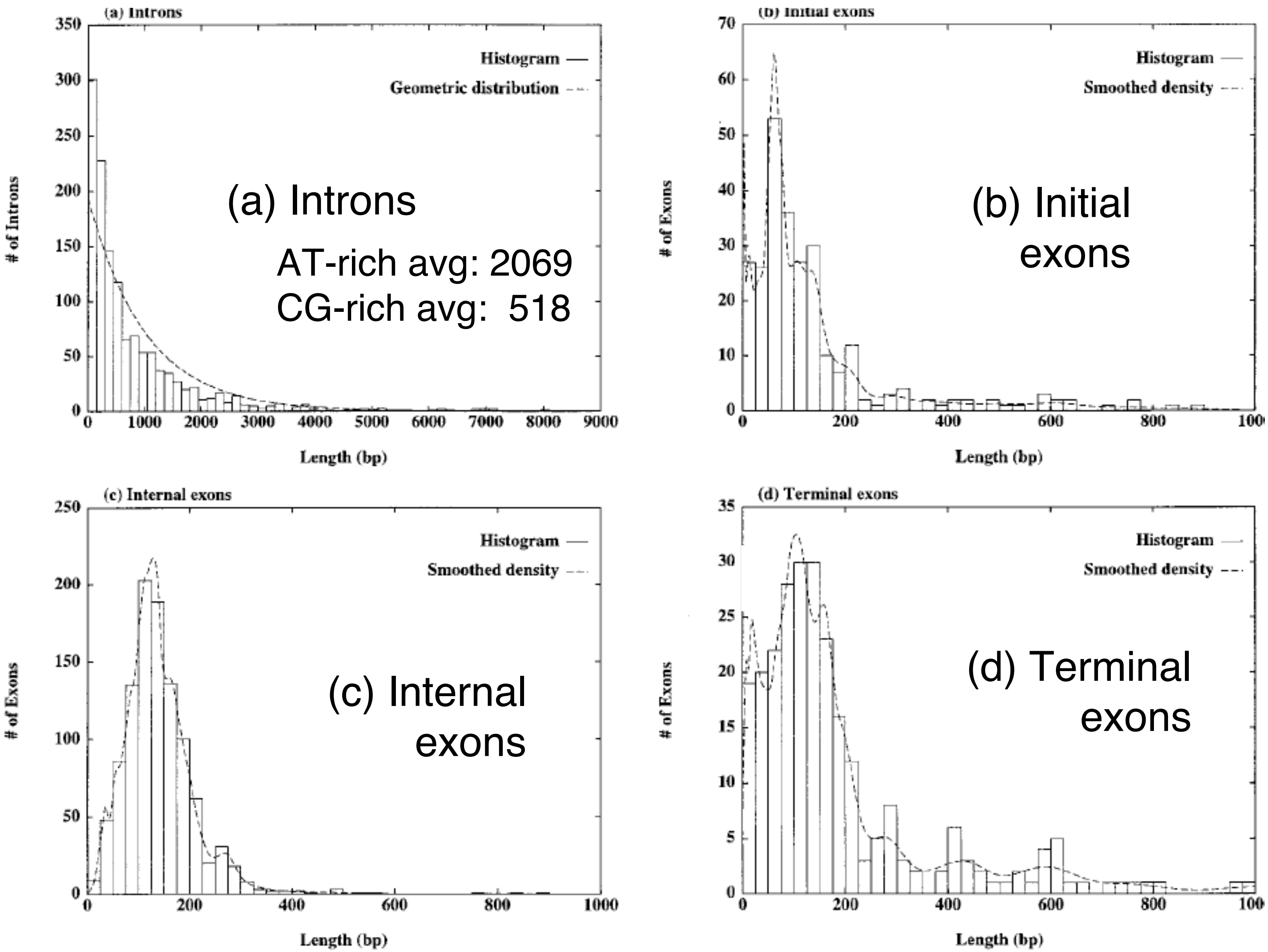


Figure 4. Length distributions are shown for (a) 1254 introns; (b) 238 initial exons; (c) 1151 internal exons; and (d) 238 terminal exons from the 238 multi-exon genes of the learning set $\mathscr{L}$. Histograms (continuous lines) were derived with a bin size of 300 bp in (a), and 25 bp in (b), (c), (d). The broken line in (a) shows a geometric (exponential) distribution with parameters derived from the mean of the intron lengths; broken lines in (b), (c) and (d) are the smoothed empirical distributions of exon lengths used by GENSCAN (details given by Burge, 1997). Note different horizontal and vertical scales are used in (a), (b), (c), (d) and that multimodality in (b) and (d) may, in part, reflect relatively

Effect of G+C Content

Group	**I**	**II**	**III**	**IV**
C ‡ G% range	<43	43-51	51-57	>57
Number of genes	65	115	99	101
Est. proportion single-exon genes	0.16	0.19	0.23	0.16
Codelen: single-exon genes (bp)	1130	1251	1304	1137
Codelen: multi-exon genes (bp)	902	908	1118	1165
Introns per multi-exon gene	5.1	4.9	5.5	5.6
Mean intron length (bp)	2069	1086	801	518
Est. mean transcript length (bp)	10866	6504	5781	4833
Isochore	L1+L2	H1+H2	H3	H3
DNA amount in genome (Mb)	2074	1054	102	68
Estimated gene number	22100	24700	9100	9100
Est. mean intergenic length	83000	36000	5400	2600
Initial probabilities:				
Intergenic (N)	0.892	0.867	0.54	0.418
Intron (I+, I-)	0.095	0.103	0.338	0.388
5' Untranslated region (F+, F-)	0.008	0.018	0.077	0.122
3' Untranslated region (T+, T-)	0.005	0.011	0.045	0.072

Submodels

- 5' UTR
  - L ~ geometric(769 bp), s ~ MM(5)
- 3' UTR
  - L ~ geometric(457 bp), s ~ MM(5)
- Intergenic
  - L ~ geometric(GC-dependent), s ~ MM(5)
- Introns
  - L ~ geometric(GC-dependent), s ~ MM(5)

Submodel: Exons

- Inhomogenious 3-periodic 5th order Markov models
- Separate models for low GC (<43%), high GC
- Track “phase” of exons, i.e. reading frame.

Signal Models I: WMM's

- Polyadenylation
  - 6 bp, consensus AATAAA
- Translation Start
  - 12 bp, starting 6 bp before start codon
- Translation stop
  - A stop codon, then 3 bp WMM

Signal Models II: more WMM's

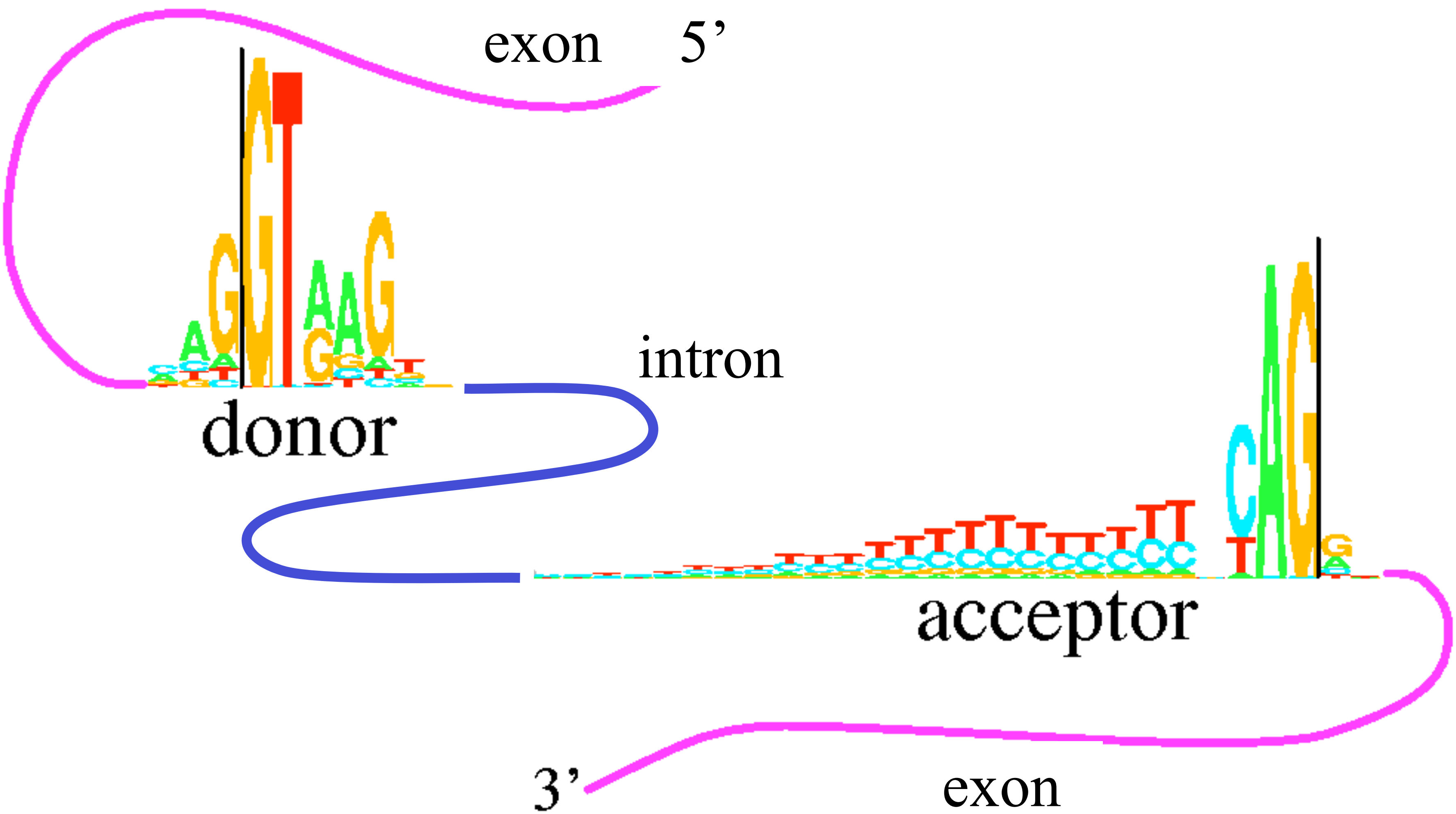
- Promoter
  - 70% TATA
    - 15 bp TATA WMM
    - s ~ null, L ~ Unif(14-20)
    - 8 bp cap signal WMM
  - 30% TATA-less
    - 40 bp null

Signal Models III: W/WAM's

- Acceptor Splice Site (3' end of intron)
  - [-20..+3] relative to splice site modeled by "1st order weight array model"
- Branch point & polypyrimidine tract
  - Hard. Even weak consensus like YYRAY found in [-40..-21] in only 30% of training
  - "Windowed WAM": 2nd order WAM, but averaged over 5 preceding positions

    "captures weak but detectable tendency toward YYY triplets and certain branch point related triplets like TGA, TAA, …"

What's in the Primary Sequence?



Signal Models IV: Maximum Dependence Decomposition

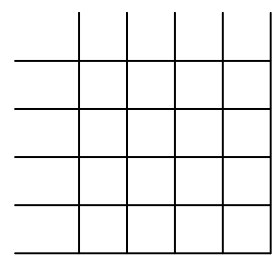
- Donor splice sites (5’ end of intron) show dependencies between non-adjacent positions, e.g. poor match at one end compensated by strong match at other end, 6 bp away
- Model is basically a decision tree
- Uses χ^2 test to quantitate dependence

χ^2 test for independence

i	Con	j:	-3	-2	-1	+3	+4	+5	+6	Sum
-3	c/a		---	61.8*	14.9	5.8	20.2*	11.2	18.0*	131.8*
-2	A		115.6*	---	40.5*	20.3*	57.5*	59.7*	42.9*	336.5*
-1	G		15.4	82.8*	---	13.0	61.5*	41.4*	96.6*	310.8*
+3	a/g		8.6	17.5*	13.1	---	19.3*	1.8	0.1	60.5*
+4	A		21.8*	56.0*	62.1*	64.1*	---	56.8*	0.2	260.9*
+5	G		11.6	60.1*	41.9*	93.6*	146.6*	---	33.6*	387.3*
+6	t		22.2*	40.7*	103.8*	26.5*	17.8*	32.6*	---	243.6*

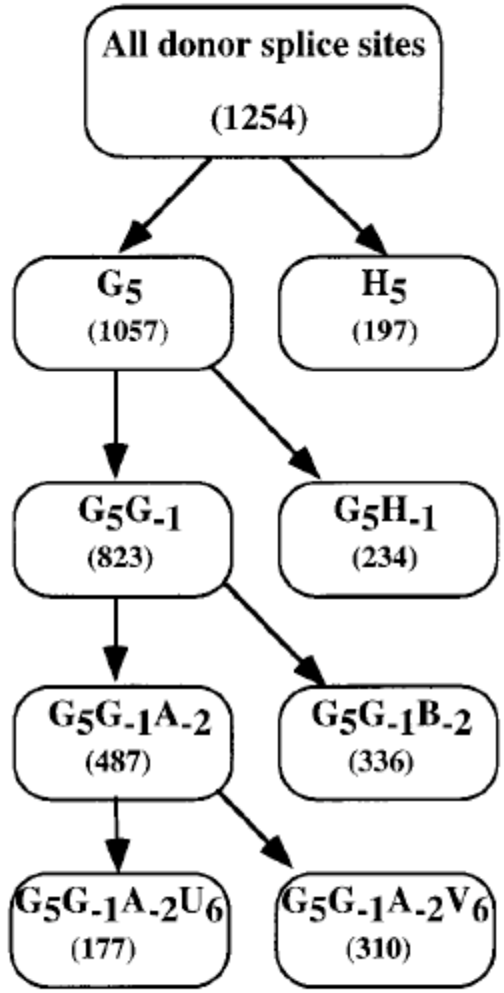
*** means chi-squared p-value < .001**

$$\chi^2 = \sum_i \frac{(\text{observed}_i - \text{expcted}_i)^2}{\text{expected}_i}$$



“expected” means expected assuming independence

Pos	A%	C%	G%	U%
-3	33	36	19	13
-2	56	15	15	15
-1	9	4	78	9
+3	44	3	51	3
+4	75	4	13	9
+6	14	18	19	49
-3	34	37	18	11
-2	59	10	15	16
+3	40	4	53	3
+4	70	4	16	10
+6	17	21	21	42
-3	37	42	18	3
+3	39	5	51	5
+4	62	5	22	11
+6	19	20	25	36
-3	32	40	23	5
+3	27	4	59	10
+4	51	5	25	19



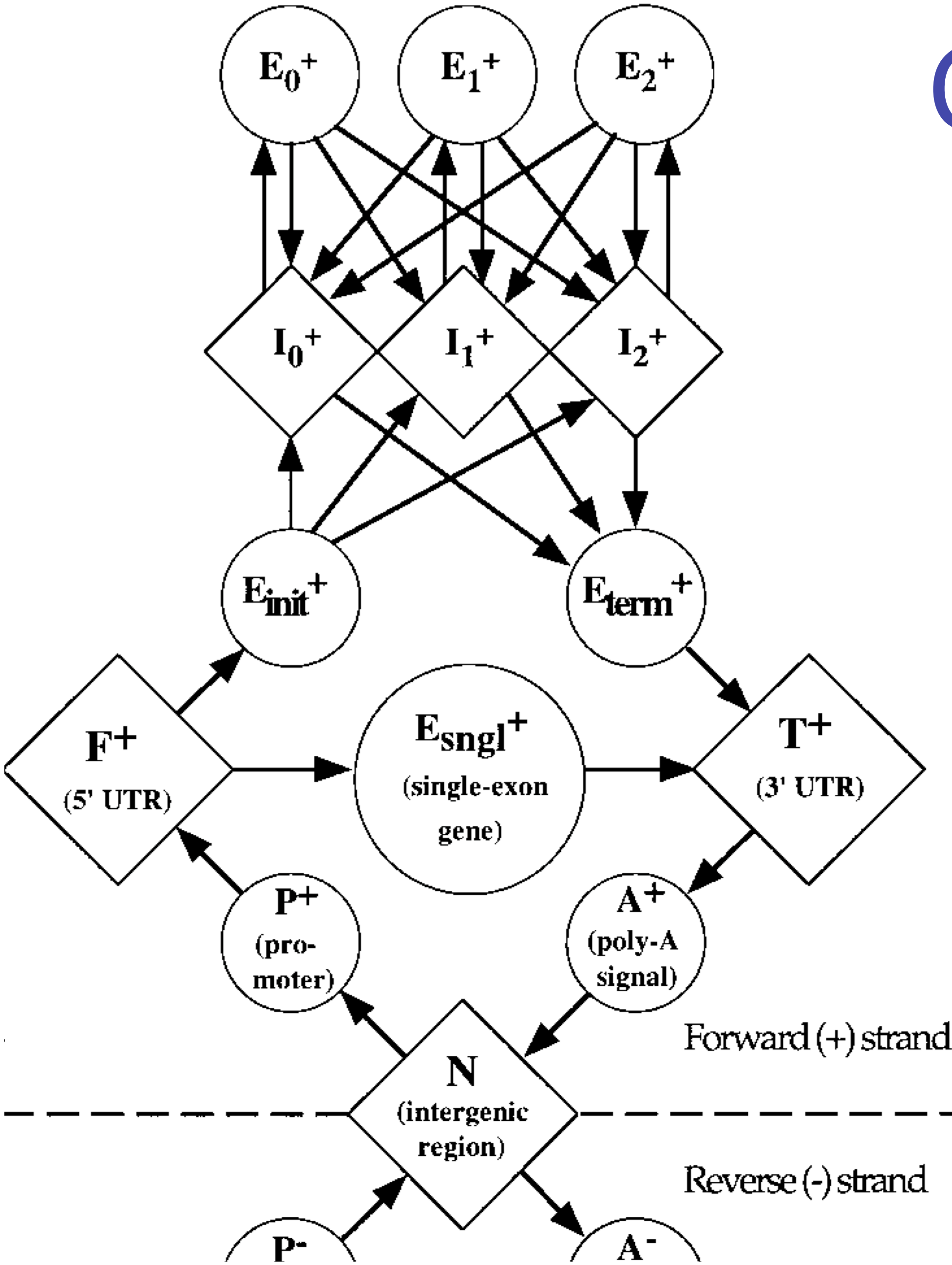
Pos	A%	C%	G%	U%
-3	35	44	16	6
-2	85	4	7	5
-1	2	1	97	0
+3	81	3	15	2
+4	51	28	9	12
+6	22	20	30	28
-3	29	31	21	18
-2	43	30	17	11
+3	56	0	43	0
+4	93	2	3	3
+6	5	10	10	76
-3	29	30	18	23
+3	42	1	56	1
+4	80	4	8	8
+6	14	21	16	49
-3	39	43	15	2
+3	46	6	46	3
+4	69	5	20	7

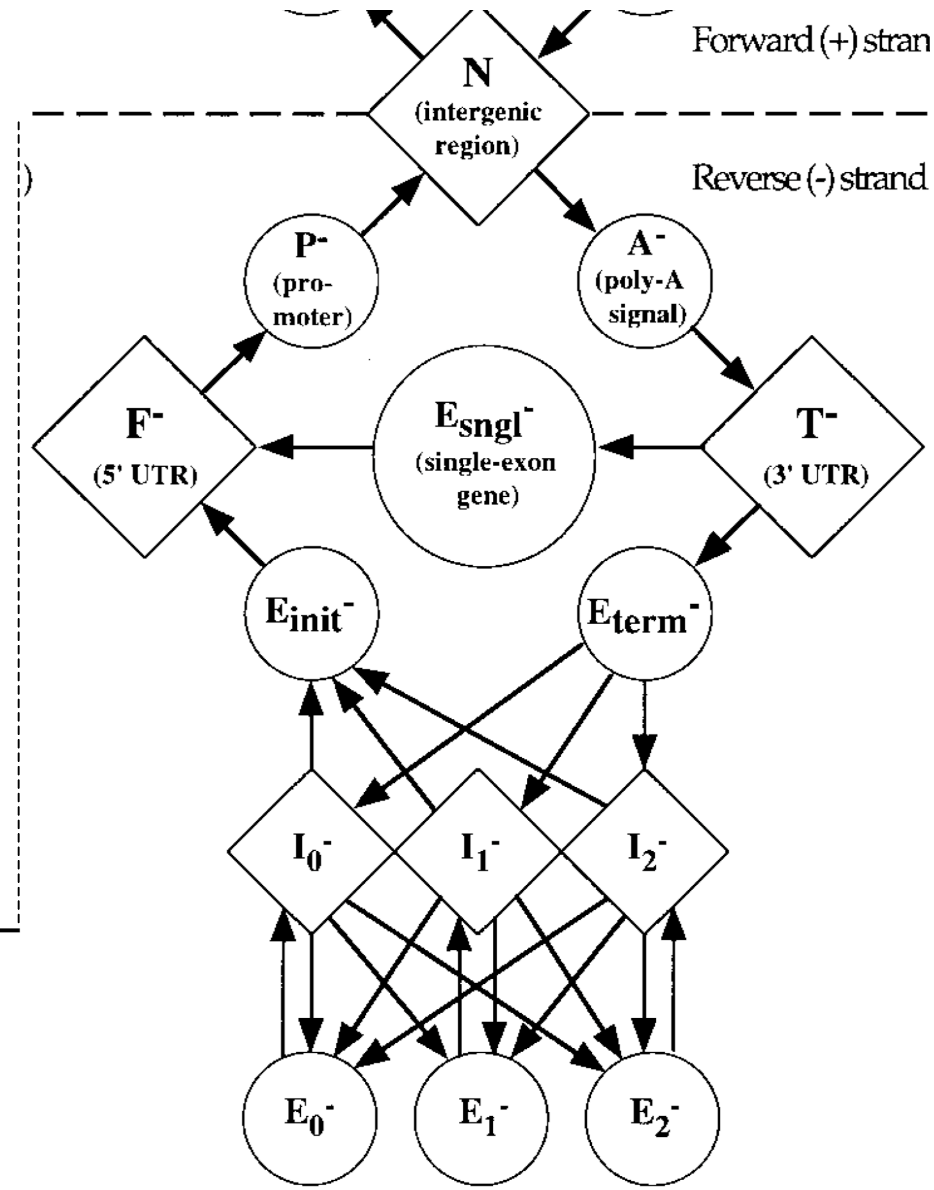
All sites: ---------- **Position** ----------

Base	**-3**	**-2**	**-1**	**+1**	**+2**	**+3**	**+4**	**+5**	**+6**
A%	**33**	**60**	8	0	0	**49**	**71**	6	15
C%	**37**	13	4	0	0	3	7	5	19
G%	18	14	**81**	**100**	0	**45**	12	**84**	20
U%	12	13	7	0	**100**	3	9	5	**46**

U1 snRNA: 3' G U C C A U U C A 5'

GHMM Structure





Summary of Burge & Karlin

- Coding DNA & control signals nonrandom
  - Weight matrices, WAMs, etc. for controls
  - Codon frequency, etc. for coding
- GHMM nice for overall architecture
- Careful attention to small details pays

Problems with BK training set

- 1 gene per sequence
- Annotation errors
- Single exon genes over-represented?
- Highly expressed genes over-represented?
- Moderate sized genes over-represented? (none > 30 kb) …
- Similar problems with other training sets, too

Problems with all methods

- Pseudo genes
- Short ORFs
- Sequencing errors
- Non-coding RNA genes & spliced UTR's
- Overlapping genes
- Alternative splicing/polyadenylation
- Hard to find novel stuff -- not in training
- Species-specific weirdness -- spliced leaders, polycistronic transcripts, RNA editing…

Other ideas

- Database search - does gene you're predicting look anything like a known protein?
- Comparative genomics - what does this region look like in related organisms?