Today’s Lecture

- Failure of equal frequency assumption
- Neutralist vs selectionist interpretations
- Site models
- Comparing models: Likelihood ratios & weight matrices

Failure of Equal Frequency Assumption for (Real) DNA

- For most organisms, the nucleotide composition is significantly different from .25 for each nucleotide, e.g.:
  - *H. influenza* .31 A, .19 C, .19 G, .31 T
  - *P. aeruginosa* .17 A, .33 C, .33 G, .17 T
  - *M. janaschii* .34 A, .16 C, .16 G, .34 T
  - *S. cerevisiae* .31 A, .19 C, .19 G, .31 T
  - *C. elegans* .32 A, .18 C, .18 G, .32 T
  - *H. sapiens* .29 A, .21 C, .21 G, .29 T

- Note approximate symmetry: A ≅ T, C ≅ G,
  - even though we’re counting nucs on just one strand.
  - Expect *exact* equality when counting both strands
- Explanation:
  - Although individual biological features may have non-symmetric composition (local *asymmetry*),
  - usually features are distributed approx *randomly* w.r.t. strand,
  - so local asymmetries *cancel*, yielding overall symmetry.

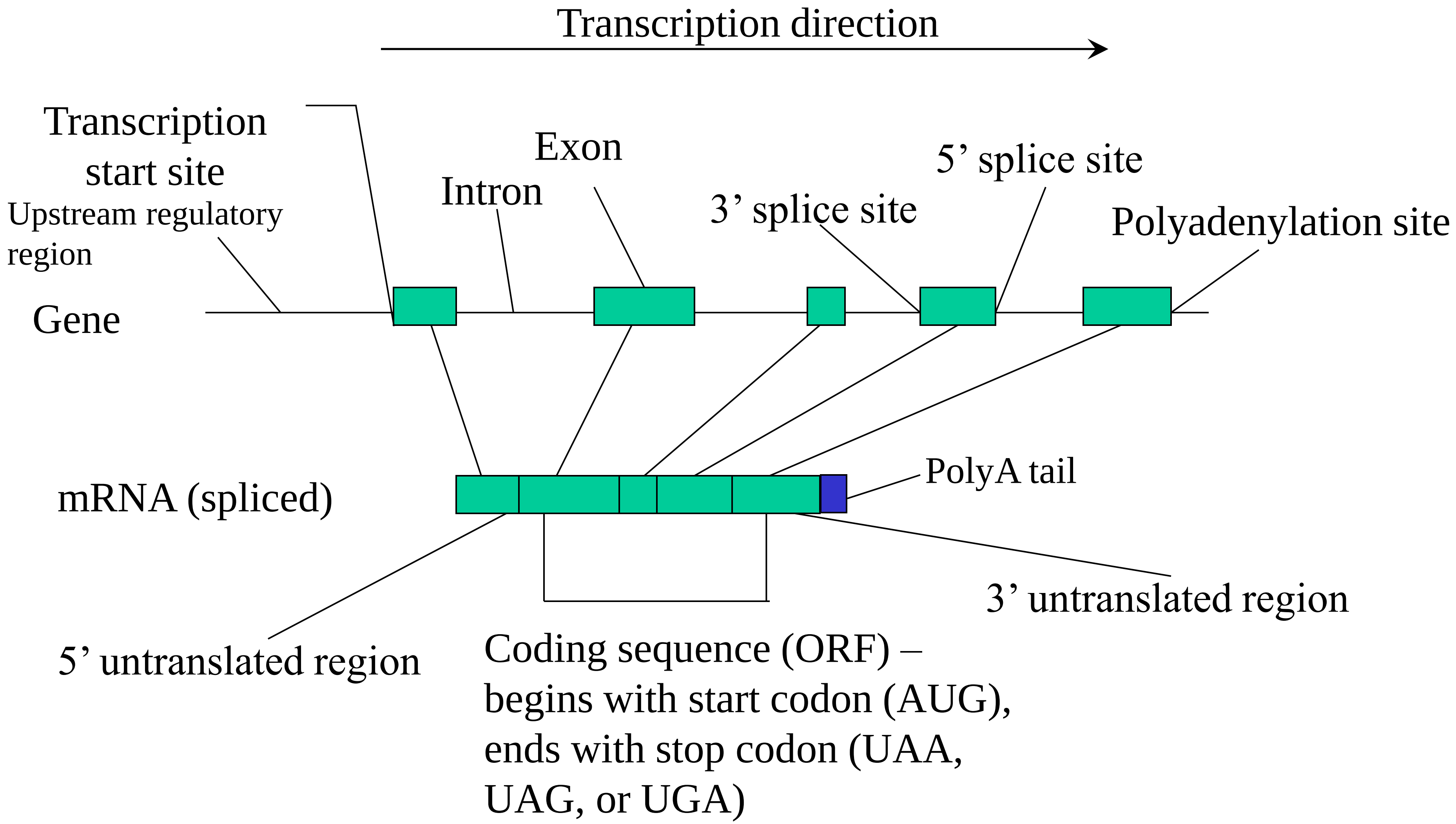
General Hypotheses Regarding Unequal Frequency

- Neutralist hypothesis: *mutation bias*
  - – e.g. due to nucleotide pool composition
- Selectionist hypothesis: *selection*
  - – selection on (many) particular nucleotides
  - – selection on mutational bias mechanisms
  - – …

Site Models

- Probability models for short sequences, such as:
  - splice sites
  - translation start sites
  - promoter elements
  - protein “motifs”

(Protein-coding) Gene Structure in Eukaryotes



Coding sequence (ORF) – begins with start codon (AUG), ends with stop codon (UAA, UAG, or UGA)

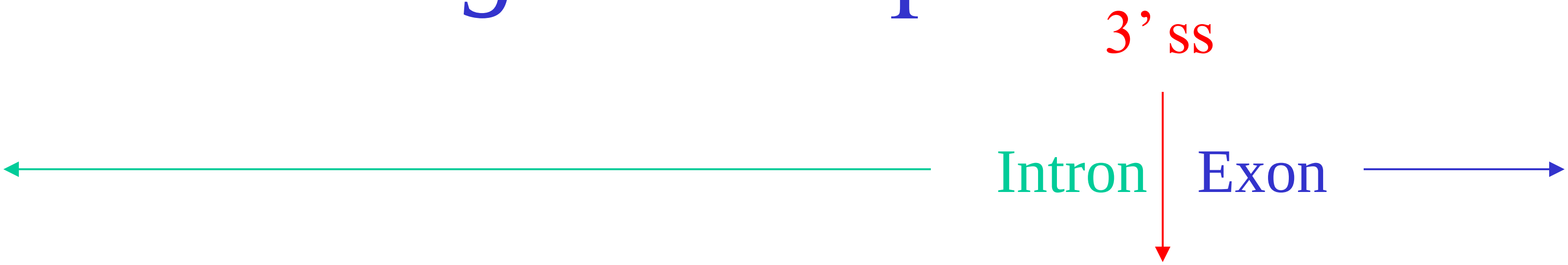
- Assumptions:
  - different examples of site can be aligned *without gaps* (indels) such that tend to have same residues in same positions
  - drop equal freq assumption: allow *position-specific freqs*
  - retain *independence* assumption (for now)

- Applies to short segments (< 30 residues) where
  - precise residue spacing is structurally or functionally important, and
  - certain positions are highly conserved
- Examples:
  - DNA/RNA sequences binding a single protein or RNA molecule
  - Protein internal regions structurally constrained due to folding requirements; or
  - protein surface regions constrained because bind certain ligands

Construction of Site Models

- Collect examples of site
- Align (without gaps)
- Count occurrences of residues at each position
- Convert to frequencies

Nucleotide Counts for 8192 *C. elegans* 3’ Splice Sites



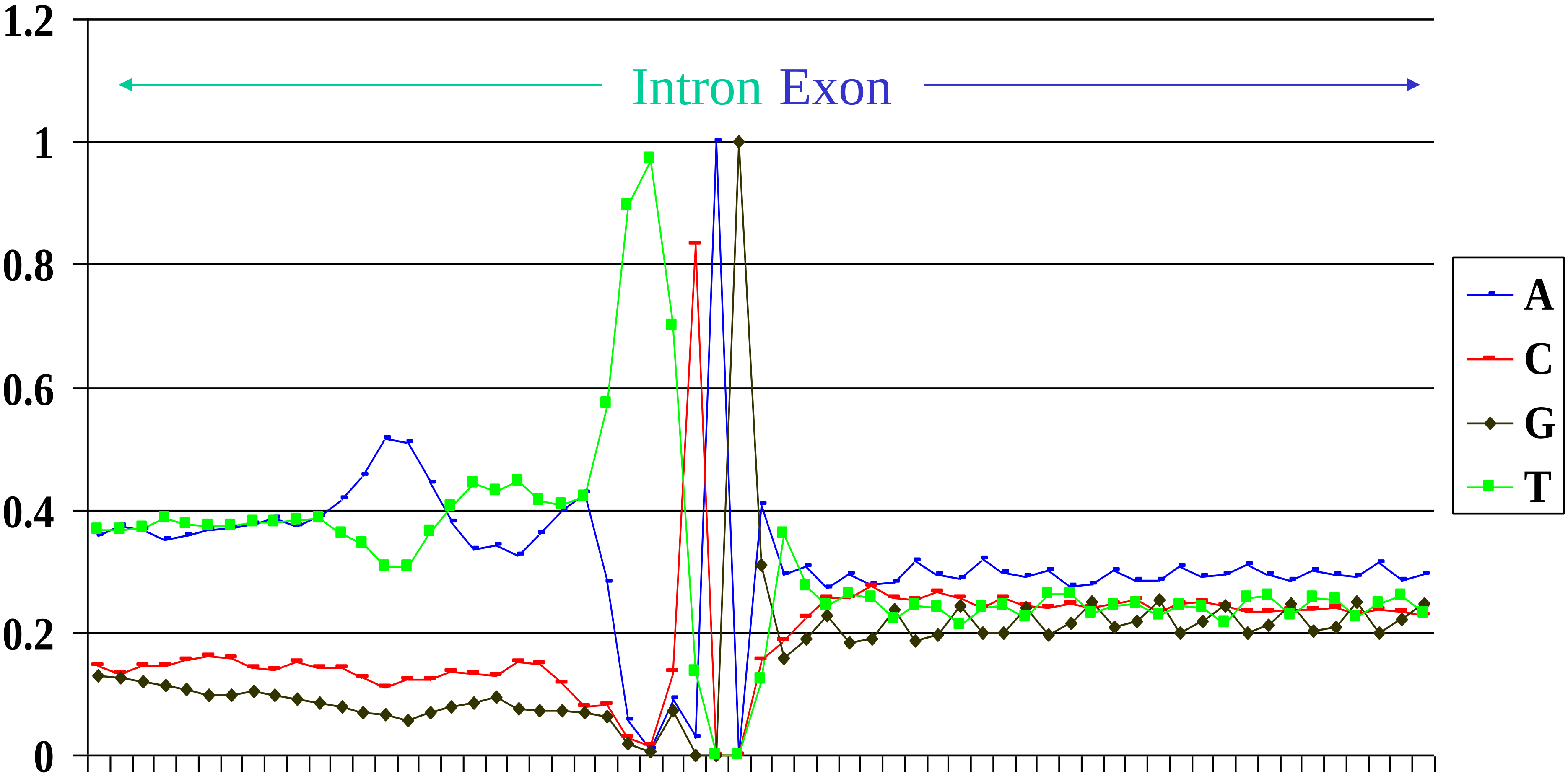
3’ ss

Intron

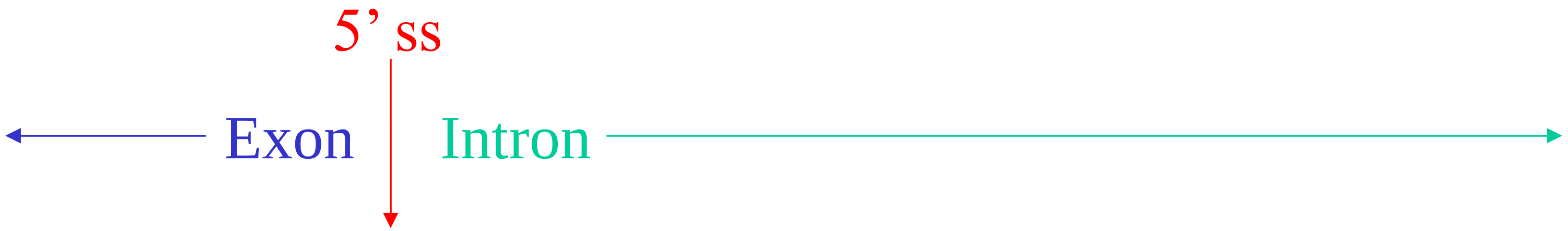
Exon

A	3276	3516	2313	476	67	757	240	8192	0	3359	2401	2514
C	970	648	664	236	129	1109	6830	0	0	1277	1533	1847
G	593	575	516	144	39	595	12	0	8192	2539	1301	1567
T	3353	3453	4699	7336	7957	5731	1110	0	0	1017	2957	2264
CONSENSUS	W	W	W	T	T	t	C	A	G	r	w	w
A	0.400	0.429	0.282	0.058	0.008	0.092	0.029	1.000	0.000	0.410	0.293	0.307
C	0.118	0.079	0.081	0.029	0.016	0.135	0.834	0.000	0.000	0.156	0.187	0.225
G	0.072	0.070	0.063	0.018	0.005	0.073	0.001	0.000	1.000	0.310	0.159	0.191
T	0.409	0.422	0.574	0.896	0.971	0.700	0.135	0.000	0.000	0.124	0.361	0.276

3’ Splice Sites – *C. elegans*



Nucleotide Counts for 8192 *C. elegans* 5’ Splice Sites



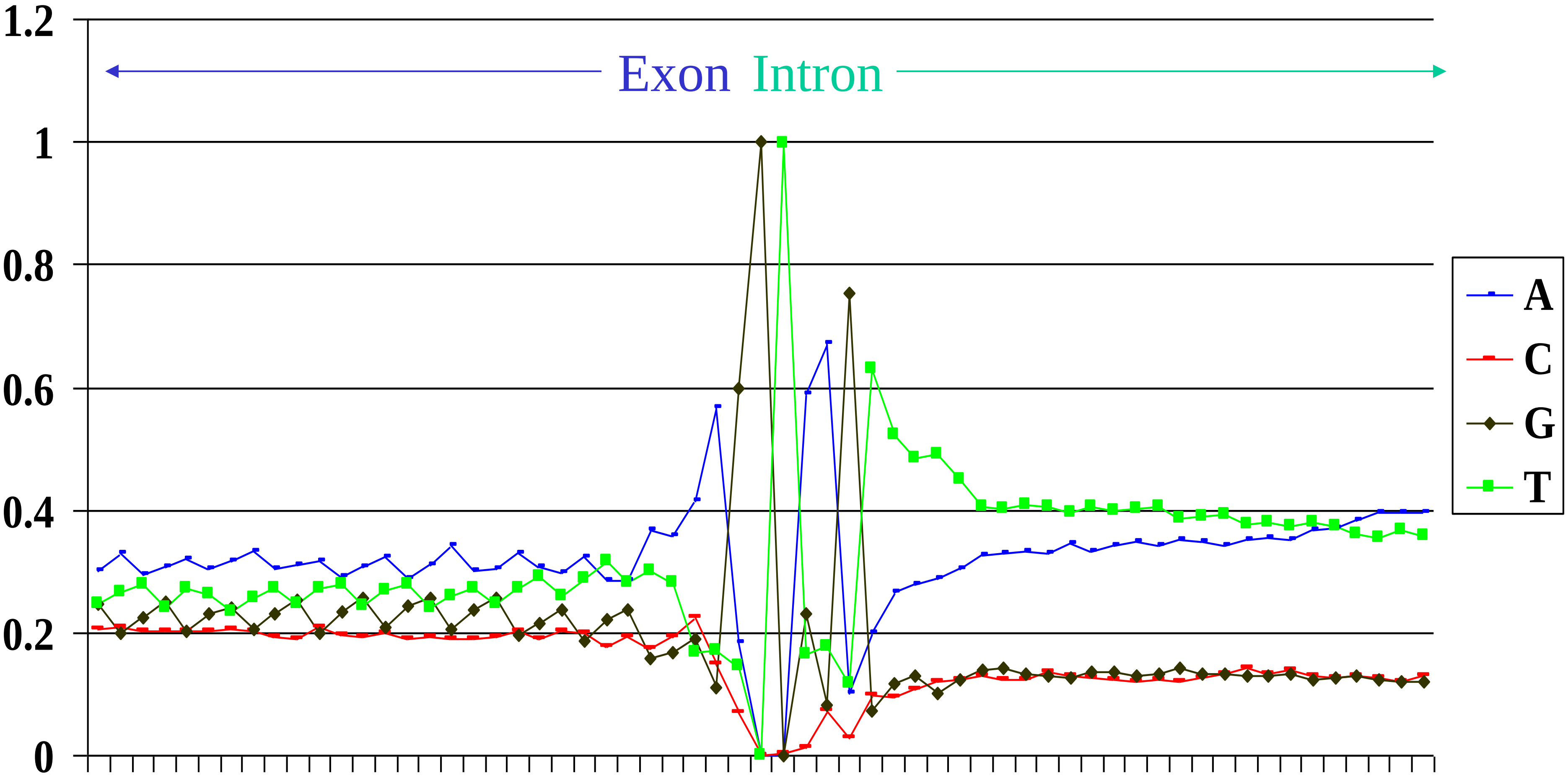
5’ ss

Exon

Intron

A	3404	4644	1518	0	0	4836	5486	837	1632	2189	2278	2355
C	1850	1224	583	0	14	118	588	237	801	771	889	986
G	1562	912	4891	8192	0	1890	672	6164	589	962	1056	827
T	1376	1412	1200	0	8178	1348	1446	954	5170	4270	3969	4024
CONSENSUS	x	a	g	G	T	a	a	g	t	t	w	t
A	0.416	0.567	0.185	0.000	0.000	0.590	0.670	0.102	0.199	0.267	0.278	0.287
C	0.226	0.149	0.071	0.000	0.002	0.014	0.072	0.029	0.098	0.094	0.109	0.120
G	0.191	0.111	0.597	1.000	0.000	0.231	0.082	0.752	0.072	0.117	0.129	0.101
T	0.168	0.172	0.146	0.000	0.998	0.165	0.177	0.116	0.631	0.521	0.484	0.491

5’ Splice Sites – *C. elegans*



Conserved Domain in RecR and Class I Topisomerases

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RecR   RLAEEKITEVILATNPTVEGEATANYIAELC
RecM   RLQDDQVTEVILATNPNIEGEATAMYISRLL
RecR   RVDDVGITEVIIATDPNTEGEATATYLVRMV
TrsI   IFKENKIDEVIIATDPAREGENIAYKILNQL
TOP1   KQLAEKADHIYLATDLDREGEAIAWRLREVI
ORF1   AELLKQANTIIVATDSDREGENIAWSIIHKA
TOP1   KDALKDADELILATDEDREGKVISWHLLQLL
TOP1   TIFDKRVKTIILATDAAAEGEYIGRNILYRL
TOP3   KREARNADYLMIWTDCDREGEYIGWEIWQEA
TOP3   KRFLHEASEIVHAGDPDREGQLLVDEVLDYL
RGYR   RNLAVEADEVLIGTDPDTEGEKIAWDLYLAL

CONSENSUS  xxxxxxxxxU&uatDxxxEGexxxxxUxxxu
```

Consensus key:

Uppercase: all residues chemically similar

lowercase: most are

U,u: bulky aliphatic (I,L,V)

&: bulky hydrophobic (I,L,V,M,F,Y,W)

From RL Tatusov, SF Altschul, and EV Koonin, PNAS 91: 12091-12095

Probability Models for Sites (assuming independence!)

- For each position i, $1 \leq i \leq n$, let P_i be a prob dist'n on the alphabet of residues
  - e.g. constructed using counts at that position in a sample of sites.
  - $P_i(r)$ for each residue r is the probability that r occurs at position i in a sequence.
- Prob dist'n P on the space S of sequences of length n is defined by

$$P(s) = \prod_{1 \leq i \leq n} P_i(s_i)$$

where $s = s_1\, s_2 \ldots s_n$

Zero Probabilities

- If $P_i(r) = 0$ for some i and r, then $P(s) = 0$ for some sequences.
  - may or may not be desirable
- If due to failure to observe residue because of small sample size,
  - should perform “small-sample correction” to change $P_i(r)$ to a small non-zero value.
  - usually done by adding ‘pseudocounts’ to each value in the counts matrix;
    - e.g. add 1 to each cell (has justification in Bayesian statistics)
  - Particularly an issue with proteins, due to larger alphabet size.
- If reflects real biological constraints
  - then leave as 0.
  - e.g. requirement for G at position +1 (first intronic base) in 5’ss

Comparing Alternative Probability Models

- We will want to consider more than one model at a time, in following situations:
  - To differentiate between two or more hypotheses about a sequence
  - To generate increasingly refined probability models that are progressively more accurate

- First situation arises in testing biological assertion, e.g. “is this a coding sequence?”
  - Compare two models:

  1. model associated with a hypothesis H_{coding},
     - assigns each sequence the prob of observing it under expt of drawing a coding sequence at random from genome
  2. model associated with a hypothesis $H_{noncoding}$,
     - assigns each sequence the prob of observing it under expt of drawing a non-coding sequence at random

Likelihood Ratios

- The *likelihood* of a model M given an observation s is

$$L(M \mid s) = P(s \mid M)$$

  This is *not* the *probability* of the model! – (the sum over all models is not 1).

- The *likelihood ratio* (LR) of two models M_a and M_0 is given by

$$LR(M_a, M_0 \mid s) = \frac{L(M_a \mid s)}{L(M_0 \mid s)}$$

  The numerator and denominator may both be very small!

- The *log likelihood ratio* (LLR) is the logarithm of the likelihood ratio.

Weight Matrices for Site Models

- LR for sites: (prob under site model) / (prob under non-site (background) model)

$$\frac{P(s \mid M_{\text{site}})}{P(s \mid M_{\text{background}})} = \frac{\prod_{1 \le i \le n} P_i(s_i \mid M_{\text{site}})}{\prod_{1 \le i \le n} P_i(s_i \mid M_{\text{background}})}$$

- LLR $= \sum_{1 \le i \le n} \log(P_i(s_i \mid M_{\text{site}})) - \log(P_i(s_i \mid M_{\text{background}}))$
  - compute by reading from a *matrix* whose *i*-th column contains values $\log(P_i(r \mid M_{\text{site}})) - \log(P_i(r \mid M_{\text{background}}))$ for each residue *r* (with *r* labelling the rows).
    - We use $\log_2$.

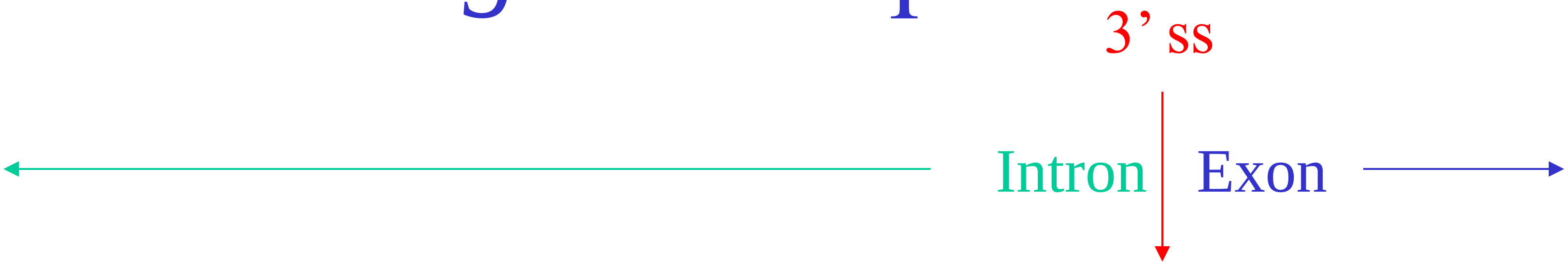
Example: 3' splice sites in *C. elegans*

- For *background distribution* take
  - genomic residue freqs computed from *C. elegans* chrom. I:

A	4,575,132:	0.321
C	2,559,048:	0.179
G	2,555,862:	0.179
T	4,582,688:	0.321

  - other choices are possible, e.g. composition of *transcribed regions*
- For the *site distribution* we take
  - site residue freqs from 8192 sites:

Nucleotide Counts for 8192 *C. elegans* 3’ Splice Sites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T	0.409	0.422	0.574	0.896	0.971	0.700	0.135	0.000	0.000	0.124	0.361	0.276

Weight Matrix – 3’ Splice Sites

```
SITE FREQUENCIES:
A  0.400  0.429  0.282  0.058  0.008  0.092  0.029  1.000  0.000  0.410  0.293  0.307
C  0.118  0.079  0.081  0.029  0.016  0.135  0.834  0.000  0.000  0.156  0.187  0.225
G  0.072  0.070  0.063  0.018  0.005  0.073  0.001  0.000  1.000  0.310  0.159  0.191
T  0.409  0.422  0.574  0.896  0.971  0.700  0.135  0.000  0.000  0.124  0.361  0.276

BACKGROUND FREQUENCIES:
A  0.321  0.321  0.321  0.321  0.321  0.321  0.321  0.321  0.321  0.321  0.321  0.321
C  0.179  0.179  0.179  0.179  0.179  0.179  0.179  0.179  0.179  0.179  0.179  0.179
G  0.179  0.179  0.179  0.179  0.179  0.179  0.179  0.179  0.179  0.179  0.179  0.179
T  0.321  0.321  0.321  0.321  0.321  0.321  0.321  0.321  0.321  0.321  0.321  0.321



WEIGHTS:
A   0.32   0.42  -0.18  -2.46  -5.29  -1.79  -3.45   1.64 -99.00   0.36  -0.13  -0.06
C  -0.60  -1.18  -1.15  -2.64  -3.51  -0.41   2.22 -99.00 -99.00  -0.20   0.06   0.33
G  -1.31  -1.35  -1.51  -3.35  -5.23  -1.30  -6.93 -99.00   2.48   0.79  -0.17   0.10
T   0.35   0.39   0.84   1.48   1.60   1.12  -1.24 -99.00 -99.00  -1.37   0.17  -0.22
```

Scoring a Candidate 3’ Splice Site

A	0.32	0.42	-0.18	-2.46	-5.29	-1.79	-3.45	1.64	-99.00	0.36	-0.13	-0.06
C	-0.60	-1.18	-1.15	-2.64	-3.51	-0.41	2.22	-99.00	-99.00	-0.20	0.06	0.33
G	-1.31	-1.35	-1.51	-3.35	-5.23	-1.30	-6.93	-99.00	2.48	0.79	-0.17	0.10
T	0.35	0.39	0.84	1.48	1.60	1.12	-1.24	-99.00	-99.00	-1.37	0.17	-0.22

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

0.35 + 0.39 +-1.15 + 1.48 + 1.60 +-1.79 + 2.22 + 1.64 + 2.48 + 0.36 +-0.13 +-0.22 = 7.23

- General def.: a *weight matrix* W has

  entries w_{rj} indexed by residues $r \in A$, and $1 \leq j \leq n$

- *score* of a sequence $s = (s_1\, s_2 \ldots s_n)$ is

$$\sum_{1 \leq j \leq n} w_{s_j j}$$

- In the site case,

$$w_{rj} = \log(P_j(r \mid M_{\text{site}})) - \log(P_j(r \mid M_{\text{background}}))$$