Some References

- A great online bib
  - http://www.nslij-genetics.org/gene/

- A good intro survey

- A gene finding bake-off
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:
  DNA \(\xrightarrow{\text{transcription}}\) RNA \(\xrightarrow{\text{translation}}\) 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

<table>
<thead>
<tr>
<th>First base</th>
<th>Second base</th>
<th>Amino-acid abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>C</td>
<td>Ala = Alanine</td>
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<tr>
<td></td>
<td></td>
<td>Arg = Arginine</td>
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<tr>
<td></td>
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<td>Asp = Aspartic acid</td>
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<tr>
<td></td>
<td></td>
<td>Asn = Asparagine</td>
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<tr>
<td></td>
<td></td>
<td>Cys = Cysteine</td>
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<td></td>
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<td>Glu = Glutamic acid</td>
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<td></td>
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<td>Gln = Glutamine</td>
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<td></td>
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<td>Gly = Glycine</td>
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<tr>
<td></td>
<td></td>
<td>His = Histidine</td>
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<tr>
<td></td>
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<td>Ile = Isoleucine</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td>Lys = Lysine</td>
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<tr>
<td></td>
<td></td>
<td>Met = Methionine</td>
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<tr>
<td></td>
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<td>Phe = Phenylalanine</td>
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<tr>
<td></td>
<td></td>
<td>Pro = Proline</td>
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<tr>
<td></td>
<td></td>
<td>Ser = Serine</td>
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<tr>
<td></td>
<td></td>
<td>Thr = Threonine</td>
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<tr>
<td></td>
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<td>Trp = Tryptophan</td>
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<td>Val = Valine</td>
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<th>Third base</th>
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</table>

- Phe = Phenylalanine
- Ser = Serine
- Tyr = Tyrosine
- Cys = Cysteine
- STOP
- Trp = Tryptophan
- 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
- Thr = Threonine
- Val = Valine
- Ala = Alanine
- Asp = Aspartic acid
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- Ile = Isoleucine
- Leu = Leucine
- Lys = Lysine
- Met = Methionine
- Phe = Phenylalanine
- Pro = Proline
- Thr = Threonine
- Val = Valine
Translation: mRNA $\rightarrow$ Protein

Watson, Gilman, Witkowski, & Zoller, 1992
Ribosomes

Watson, Gilman, Wilkowski, & 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 = $\frac{64}{3} = 21$ triplets
  - 300bp ORF once per 36kbp per strand
- But average protein $\sim$ 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. $f(abc)$
  - $a_1a_2a_3a_4...a_{3n+2}$ is coding, unknown frame

- Calculate
  - $p_1 = f(a_1a_2a_3)f(a_4a_5a_6)...f(a_{3n-2}a_{3n-1}a_{3n})$
  - $p_2 = f(a_2a_3a_4)f(a_5a_6a_7)...f(a_{3n-1}a_{3n}a_{3n+1})$
  - $p_3 = f(a_3a_4a_5)f(a_6a_7a_8)...f(a_{3n}a_{3n+1}a_{3n+2})$
  - $P_i = p_i / (p_1+p_2+p_3)$

- More generally: k-th order Markov model
  - k=5 or 6 is typical
Codon Usage in Φx174
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

\[
\begin{array}{cccc}
5' & \text{exon} & \text{intron} & \text{exon} & \text{intron} \\
\text{AG/GT} & \text{yyy..AG/G} & \text{AG/GT} \\
\text{donor} & \text{acceptor} & \text{donor}
\end{array}
\]
## Characteristics of human genes
*(Nature, 2/2001, Table 21)*

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

*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
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

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

<table>
<thead>
<tr>
<th>Program</th>
<th>Sensitivity per nuc.</th>
<th>Specificity per nuc.</th>
<th>Sensitivity per exon</th>
<th>Specificity per exon</th>
<th>Accuracy per exon</th>
<th>ME</th>
<th>WE</th>
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<tbody>
<tr>
<td>GENSSCAN</td>
<td>0.93</td>
<td>0.93</td>
<td>0.78</td>
<td>0.81</td>
<td>0.8</td>
<td>0.09</td>
<td>0.05</td>
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<td>FGENEH</td>
<td>0.77</td>
<td>0.88</td>
<td>0.61</td>
<td>0.64</td>
<td>0.64</td>
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<td>0.46</td>
<td>0.45</td>
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<td>Genie</td>
<td>0.76</td>
<td>0.77</td>
<td>0.55</td>
<td>0.48</td>
<td>0.51</td>
<td>0.17</td>
<td>0.33</td>
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<td>GenLang</td>
<td>0.72</td>
<td>0.79</td>
<td>0.51</td>
<td>0.52</td>
<td>0.52</td>
<td>0.21</td>
<td>0.22</td>
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<tr>
<td>GeneParser2</td>
<td>0.66</td>
<td>0.79</td>
<td>0.35</td>
<td>0.4</td>
<td>0.37</td>
<td>0.34</td>
<td>0.17</td>
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<tr>
<td>GRAIL2</td>
<td>0.72</td>
<td>0.87</td>
<td>0.36</td>
<td>0.43</td>
<td>0.4</td>
<td>0.25</td>
<td>0.11</td>
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<td>SORFIND</td>
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<td>0.85</td>
<td>0.42</td>
<td>0.47</td>
<td>0.45</td>
<td>0.24</td>
<td>0.14</td>
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<td>Xpound</td>
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<td>0.87</td>
<td>0.15</td>
<td>0.18</td>
<td>0.17</td>
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<td>0.13</td>
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<td>GeneID‡</td>
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<td>0.91</td>
<td>0.73</td>
<td>0.7</td>
<td>0.71</td>
<td>0.07</td>
<td>0.13</td>
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<td>GeneParser3</td>
<td>0.86</td>
<td>0.91</td>
<td>0.56</td>
<td>0.58</td>
<td>0.57</td>
<td>0.14</td>
<td>0.09</td>
</tr>
</tbody>
</table>

After Burge&Karlin, Table 1. Sensitivity $Sn = TP/AP$; Specificity, $Sp = TP/PP$
Generalized Hidden Markov Models

- $\pi$: 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 $d_i = |s_i|$ $\sim$ submodel for $q_i$
    - Pick next state $q_{i+1}$ ($\sim a_{ij}$)
  - Output $s_1s_2...$
"Parse" a $S = S_1 S_2 \cdots S_k$
is $d_1 d_2 \cdots d_k$ s.t. $\Sigma d_i = L$
& $2 \leq L \leq \delta k$

$Pr (\Phi (S) = \frac{Pr (\Phi (S))}{Pr (S)}$}

\[ \vdots \]

E.g. Use something like forward/backward
to code. Prove that positions $i \cdots j$
are an atom of phase $k$ in $S$. 

23
GHMM Structure
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 $\mathcal{F}$. 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

<table>
<thead>
<tr>
<th>Group</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
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<tr>
<td>C ± G% range</td>
<td>&lt;43</td>
<td>43-51</td>
<td>51-57</td>
<td>&gt;57</td>
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<tr>
<td>Number of genes</td>
<td>65</td>
<td>115</td>
<td>99</td>
<td>101</td>
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<tr>
<td>Est. proportion single-exon genes</td>
<td>0.16</td>
<td>0.19</td>
<td>0.23</td>
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<td>Codelen: single-exon genes (bp)</td>
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<td>1251</td>
<td>1304</td>
<td>1137</td>
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<tr>
<td>Codelen: multi-exon genes (bp)</td>
<td>902</td>
<td>908</td>
<td>1118</td>
<td>1165</td>
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<tr>
<td>Introns per multi-exon gene</td>
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<td>4.9</td>
<td>5.5</td>
<td>5.6</td>
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<tr>
<td>Mean intron length (bp)</td>
<td>2069</td>
<td>1086</td>
<td>801</td>
<td>518</td>
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<tr>
<td>Est. mean transcript length (bp)</td>
<td>10866</td>
<td>6504</td>
<td>5781</td>
<td>4833</td>
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<tr>
<td>Isochore</td>
<td>L1+L2</td>
<td>H1+H2</td>
<td>H3</td>
<td>H3</td>
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<td>DNA amount in genome (Mb)</td>
<td>2074</td>
<td>1054</td>
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<td>9100</td>
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<tr>
<td>Est. mean intergenic length</td>
<td>83000</td>
<td>36000</td>
<td>5400</td>
<td>2600</td>
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### Initial probabilities:

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<th>II</th>
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<td>Intergenic (N)</td>
<td>0.892</td>
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<td>0.54</td>
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<td>Intron (I+, I-)</td>
<td>0.095</td>
<td>0.103</td>
<td>0.338</td>
<td>0.388</td>
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<tr>
<td>5' Untranslated region (F+, F-)</td>
<td>0.008</td>
<td>0.018</td>
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<tr>
<td>3' Untranslated region (T+, T-)</td>
<td>0.005</td>
<td>0.011</td>
<td>0.045</td>
<td>0.072</td>
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</table>
Submodels

- 5’ UTR
  - $L \sim \text{geometric}(769 \text{ bp}), \ s \sim \text{MM}(5)$
- 3’ UTR
  - $L \sim \text{geometric}(457 \text{ bp}), \ s \sim \text{MM}(5)$
- Intergenic
  - $L \sim \text{geometric}(\text{GC-dependent}), \ s \sim \text{MM}(5)$
- Introns
  - $L \text{ from empirical distribution}, \ s \sim \text{MM}(5)$
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, …”
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 $\chi^2$ test quantitate dependence
**χ² test for independence**

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<tr>
<th>i</th>
<th>Con</th>
<th>j:</th>
<th>-3</th>
<th>-2</th>
<th>-1</th>
<th>+3</th>
<th>+4</th>
<th>+5</th>
<th>+6</th>
<th>Sum</th>
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<td>14.9</td>
<td>5.8</td>
<td>20.2*</td>
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<td>131.8*</td>
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<td>115.6*</td>
<td>---</td>
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<td>20.3*</td>
<td>57.5*</td>
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<td>61.5*</td>
<td>41.4*</td>
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* means chi-squared p-value < .001

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

“expected” means expected assuming independence
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All sites:

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