Today's Lecture

- PhastCons
- Karlin-Altschul theory

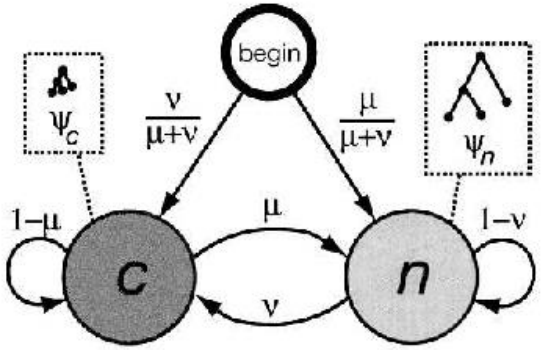
Notation

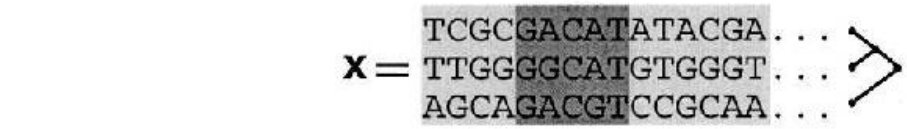
- $\mu = a_{cn}$, $\omega = 1/\mu$ (expected length of conserved elt)
- $\nu = a_{nc}$
- expected 'coverage' γ (frac of genome that is conserved):

  = Elen (cons seg) / (Elen(cons seg) + (Elen(neut seg))

  = $(1/\mu) / (1/\mu + 1/\nu)$

  = $\nu / (\mu + \nu)$





Instead: -- impose constraints

- coverage constraint:
  - 65% of coding bases covered by conserved elts
  - (target value based on earlier mouse/human analysis)
- smoothness constraint:
  - PIT (≡ expected min. amt of phylogenetic info required to predict a conserved element)

    = 9.8 bits
    - (forced to be same for all species groups)

- constraints met by ‘tuning’ γ and ω (or equivalently transit probs)
  - choose γ and ω,
  - get ML estimates of other parameters by EM algorithm
  - see whether get desired coverage & PIT
  - if not, adjust γ and ω & redo

- $L_{\min}$: expected min length of a conserved segment that could appear in a Viterbi path
- at $L_{\min}$,

  expected loglike of staying in state n

  = expected loglike of switching to c & back again, so

$$(L_{\min}+1)\log(1-\nu)+L_{\min}\sum_x P(x|\psi_c)\log P(x|\psi_n)$$

$$=\log\nu+\log\mu+(L_{\min}-1)\log(1-\mu)+L_{\min}\sum_x P(x|\psi_c)\log P(x|\psi_c)$$

- $$L_{\min}=\frac{\log\nu+\log\mu-\log(1-\nu)-\log(1-\mu)}{\log(1-\nu)-\log(1-\mu)-H(\psi_c\|\psi_n)}$$

- where

$$H(\psi_c||\psi_n) = \sum_x P(x|\psi_c) \log \frac{P(x|\psi_c)}{P(x|\psi_n)}$$

= rel entropy of *c*-state emission prob dist'n w.r.t.

n-state dist'n

- PIT (phylogenetic information threshold)

= $L_{\min} H(\psi_c||\psi_n)$

= 'expected min amt of phylogenetic info required to predict conserved element'

- Final param estimates (for vertebrates):
  - $\gamma = 0.265$
  - $\omega = 12.0$ bp
  - $H(\psi_c || \psi_n) = .608$ bits / site
  - $L_{min} = 16.1$ bp
  - PIT = $L_{min} H(\psi_c || \psi_n) = 9.8$ bits

Group	Method	Total no.[a]	Ave. len.[b]	Cov.[c]	CDS cov.[d]	μ	ν	ω	γ	$L_{\min}$
vert.	MLE	561,103	216.1	4.2%	68.8%	0.018	0.004	55.4	0.191	30.4
	55%	1,058,855	75.3	2.8%	56.8%	0.125	0.029	8.0	0.187	12.9
	65%[e]	1,157,180	103.5	4.2%	66.1%	0.083	0.030	12.0	0.265	16.0
	75%	1,381,978	167.5	8.1%	76.6%	0.043	0.031	23.0	0.415	22.6

Group	Method	Total no.[a]	Ave. len.[b]	Cov.[c]	CDS cov.[d]	CDS frac.[e]	$H(\psi_c \| \psi_n)$	$L_{\min}$
vert.	65%	1,157,180	103.5	4.2%	66.1%	18.0%	0.611	16.0
	4d	797,777	109.3	3.0%	64.2%	24.0%	0.854	11.0

Estimating false positive rates

- simulate 1 Mb alignment
  - by sampling 4D sites (with replacement) from aligned CDSs
  - caveat: these not typical of all neutral sites!
- predict cons elts (using prev param estimates)
- frac of bases in cons elts:

Group	65%	75%	MLE
vertebrate	0.00279[a]	0.00362	0.00005
insect	0.00286	0.01026	0.00152
worm	0.00000	0.00000	0.00000
yeast	0.00006	0.00042	0.00023

- does not address (important) issue of rate of false positive bases within, or flanking, true conserved elements
- also: genes more G+C rich than genome average, & have somewhat higher mutation rate (due in part to more frequent CpGs)

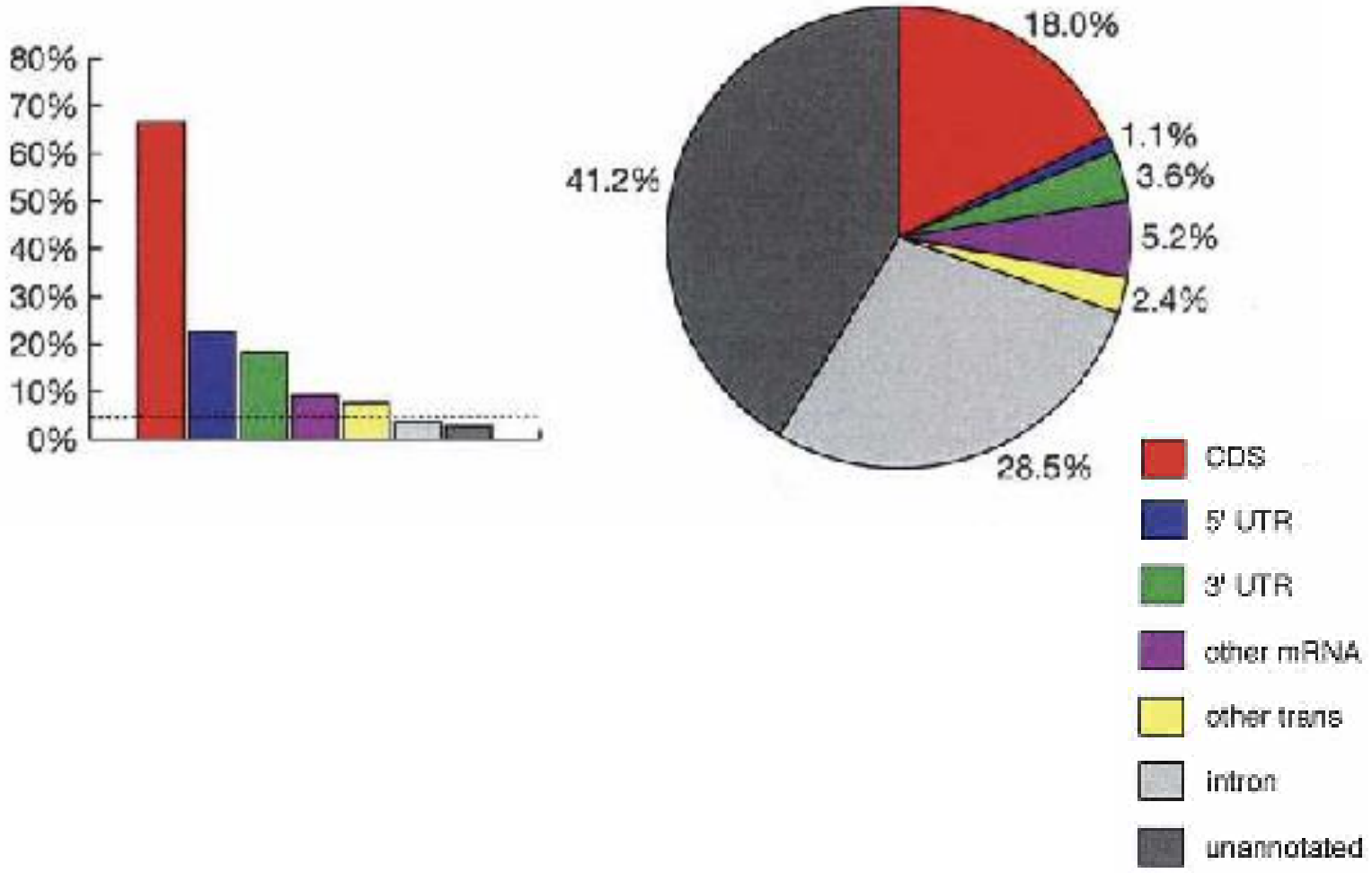
  $\Rightarrow$ *underestimating* false pos rate
- also: randomization procedure destroys underlying mutation rate variation

  $\Rightarrow$ *underestimating* false pos rate

Characteristics of phastCons predicted conserved elements

- 1.18 million elements
- constitute 4.3% of human sequence
  - 66% of coding bases
    - 88% of coding exons overlap predicted elt
  - 23% of 5'UTR bases
    - 63% of exons
  - 18% of 3'UTR bases
    - 64% of exons
  - 42% of RNA gene bases
    - 56% of genes
  - 3.6% of intronic bases
  - 2.7% of intergenic bases
  - < 1% of mammalian 'ancestral repeats' (ARs)

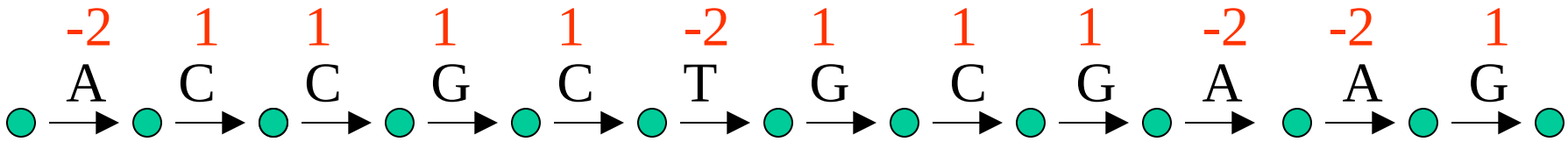
Vertebrate
(H. sapiens)



from Siepel A. *et al.* (2005). Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res.* 15:1034-50.

Context for Karlin-Altschul Theory for Maximal Segment Analysis

- Linked list, with labels attached to edges, e.g.
  - a sequence graph: labels = sequence residues
  - (ungapped) aligned pair of seqs: labels = possible alignment columns (pairs of residues)
- edge weights depend only on labels:
  - each label is assigned a weight $W(s) = w_s$



- in backgd model, each label *s* occurs with probability $P(s) = p_s$ where
  - P = prob dist'n on sample space S = {labels}

Methods for Computing Statistical Significance of Maximal Segment Scores

1. exact prob dist'n
2. approximate formula (Karlin-Altschul)
3. from simulated sequences
4. from real biological 'background' sequences
   - i.e. not having feature in question

1, 2, 3 require prob model approximating biological reality; 4 requires an appropriate dataset

2 is faster than 1 or 3, but involves add'l approximations (ignores 'edge effects')

1 requires more complex algorithm

Exact Score Dist'n for Segments in WLLs

- Exact score dist'n (following proof allows position-specific scores and probabilities):
  - Let $P_{k,m}^{(i)}$ = prob that :
    - highest-scoring path *ending at position i* has score *k*, *and also*
    - highest scoring path *ending at any pos'n* $\leq i$ has score *m*
  - special cases:
    - $P_{k,m}^{(i)} = 0$ if $k < 0$ or $m < k$;
    - $P_{0,0}^{(0)} = 1$,
    - $P_{k,m}^{(0)} = 0$ if k or $m \neq 0$
  - dist'n of maximum score is $P_m = \sum_{k \leq m} P_{k,m}^{(N)}$.
    (N = seq length)

- Algorithm to compute $\{P_{k,m}^{(i)}\}$ from $\{P_{k,m}^{(i-1)}\}$:
  - If $0 < k < m$
    - ($\Rightarrow$ best path ending at position i cannot start at i, and best path ending at position $\leq i - 1$ must have score = m)

    then $P_{k,m}^{(i)} = \sum_j p_j^{(i)} P_{k-j,m}^{(i-1)}$
  - if $0 < k = m$
    - ($\Rightarrow$ best path ending at position $\leq i - 1$ may have score $\leq m$)

    then $P_{k,m}^{(i)} = \sum_j p_j^{(i)} \sum_{n \leq m} P_{k-j,n}^{(i-1)}$
  - $P_{0,m}^{(i)} = \sum_j p_j^{(i)} \sum_{n \leq -j} P_{n,m}^{(i-1)}$
  - stop when i reaches N

- Can incorporate Markov chain dependencies in sequence probs:
  - just keep track of preceding residue r as well as k,m : $P_{r,k,m}^{(i)}$.
- Reduce required memory by truncating for large m, with appropriate modifications.
- Would like to have generalization to arbitrary DAG (e.g. edit graphs for sequence alignment)!
  - Difficult, because $P_{k,m}^{(v)}$ not independent for different parent vertices v

Why Is *Approximation* to Exact Score Distribution of Interest?

- faster to compute: useful for database searches
- gives better intuition for score behavior
- *Form* of approximation extends to other situations
  - e.g. gapped alignments

  where exact dist'n currently unavailable

Approximate Score Distribution for High-Scoring Segments in WLLs: Karlin-Altschul theory

- Main reason why BLAST is most widely used computational biology tool!
- Ideas closely related to
  - classical random walk and gambler's ruin problems in probability theory
    - (cf. W. Feller, *An Introduction to Probability Theory and Its Applications*),
  - sequential sampling in statistics