Lecture 17

- Parsing genomes with HMMs

Genome biology overview

- Genomes undergo two fundamental processes (both involve copying!):
  - Replication
  - Transcription
- Genomic functional information is in the form of *sites:*
  - Short (~2 – ~15 base) sequence segments that bind to an *RNA* or *protein* molecule (the *reader*) to help mediate some function

Genome HMMs

- a genome consists of (functionally important) ***sites*** within (nonfunctional) ***background*** sequence.
- can define an HMM that reflects this:
  - one state per site position, for each type of site
  - background state
  - appropriate topology (allowed transitions)
  - emission & transition probs

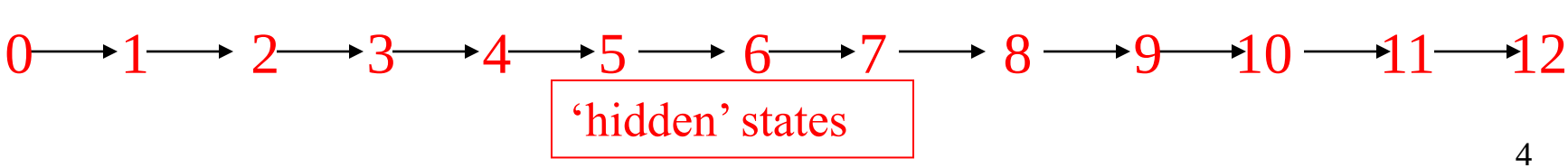
and use it to get Viterbi parse & posterior probs

HMM for *C. elegans* 3' Splice Sites

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

Emission probabilities

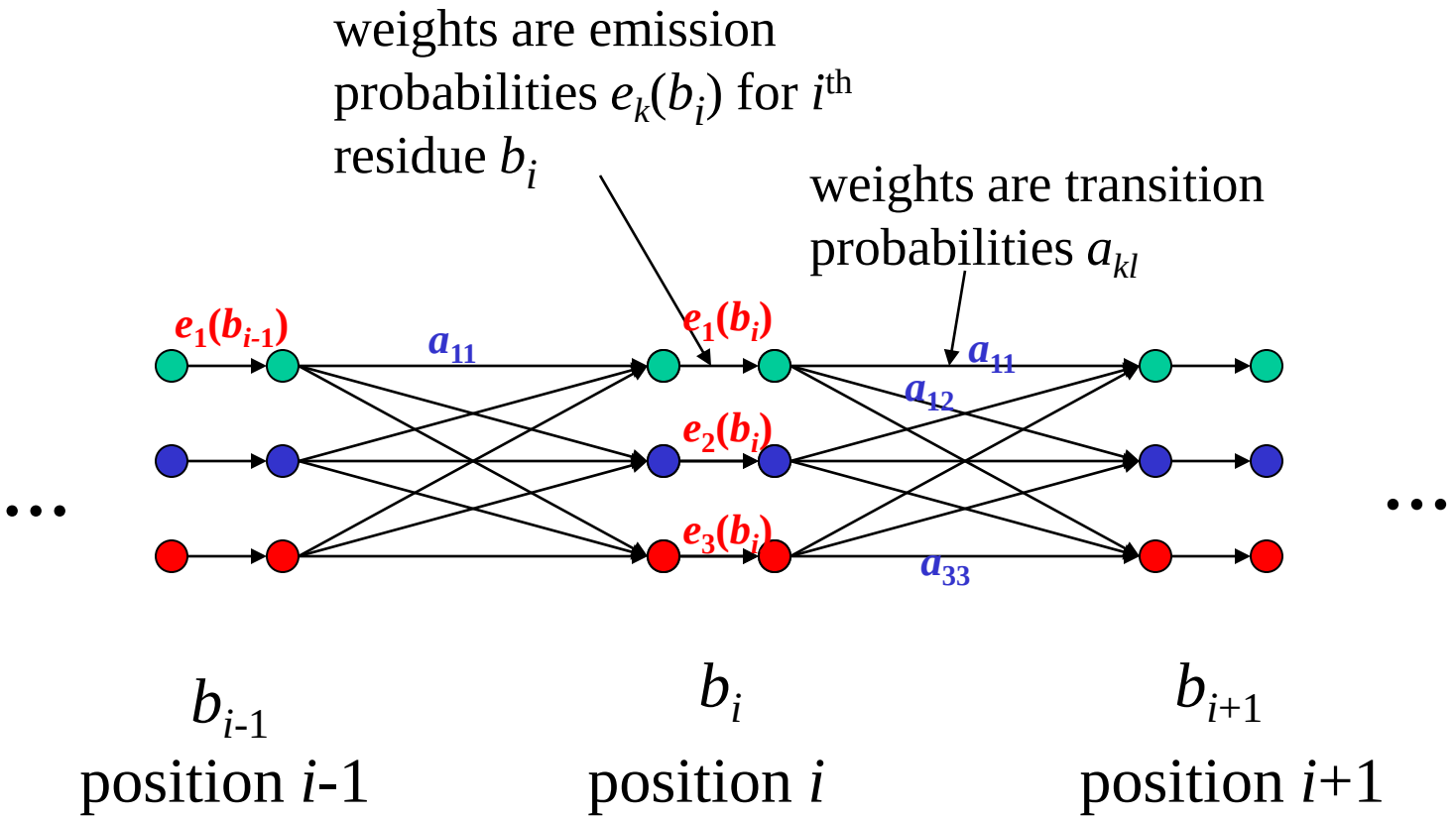
A	0.400	0.429	0.282	0.058	0.008	0.092	0.029	1.000	0.000	0.410	0.293	0.307
C	0.118	0.079	0.081	0.029	0.016	0.135	0.834	0.000	0.000	0.156	0.187	0.225
G	0.072	0.070	0.063	0.018	0.005	0.073	0.001	0.000	1.000	0.310	0.159	0.191
T	0.409	0.422	0.574	0.896	0.971	0.700	0.135	0.000	0.000	0.124	0.361	0.276



- Complication: sites have ***orientation*** (top or bottom ***strand***)
  - e.g. from transcription direction
- One strategy: analyze 2 strands separately
  - problem: resolving conflicts
- Better strategy: ***expand model*** to allow sites in both orientations, and run on ***top strand*** only
  - double # site states
  - bottom strand states have
    - complementary emission probs
    - reversed allowed transitions

- # params does not change
- size of WDAG increases, but only by factor of ~2
  - no transitions *between* top & bottom strand states, except for background

cf. WDAG for 3-state HMM length n sequence (lecture 13)



Prokaryotes vs eukaryotes

- Such HMMs are most reliable, & most widely used, for prokaryotic genomes, which usually have
  - high site density, homogeneous background
  - relatively simple spatial relationships among sites
  - often relatively little ‘supporting information’ such as
    - protein binding & transcript data
    - closely related genomes

- eukaryotic genomes are less suitable:
  - low site density, heterogeneous background
  - complex site spatial relationships (not well captured by Markov transition model)
  - often much supporting info
    - similar genomes to transfer annotations from;
    - protein binding / RNASeq & other experimental data
    - in principle, some of this could be incorporated into HMM
      - (expanded symbol alphabet)

Prokaryote genomes

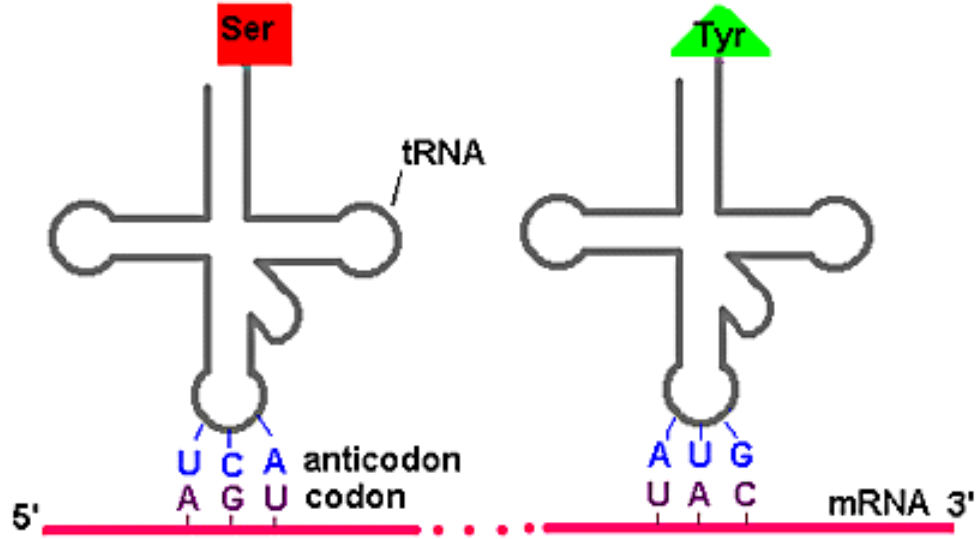
- typically a few MB in size
- up to ~80% protein coding
- Typical CDS size ~1 KB
- introns & overlapping CDSs rare
- range of GC contents

ORF analysis

- Translate genome in all 6 reading frames
- In each, find ‘open reading frames’ starting with ATG (or NTG), ending in stop
- Sort ORFs by (decreasing) length
- Work through sorted list, discarding any ORF that
  - overlaps a longer one, or
  - is ‘too short’

- Problems:
  - short CDSs are missed
  - CDSs often have long overlapping fake ORFs on opposite strand
  - poor performance on GC-rich genomes (many long fake ORFs

- Additional information that is present in real coding sequence (but ignored in ORF analysis) – *cf. lecture 3*
  - amino acid usage
  - synonymous codon bias
- Use this, in a probability model!



2nd base in codon

1st base in codon	U	C	A	G	3rd base in codon
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	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

The Genetic Code

Amino Acid	Obs/Exp	1st codon base	2nd codon base	3rd codon base	# codons
E	1.92	G	A	R	2
K	1.80	A	A	R	2
D	1.62	G	A	Y	2
M	1.46	A	T	G	1
N	1.37	A	A	Y	2
F	1.25	T	T	Y	2
Q	1.22	C	A	R	2
I	1.16	A	T	Not G	3
A	1.14	G	C	N	4
G	1.05	G	G	N	4
V	.99	G	T	N	4
Y	.98	T	A	Y	2
L	.95	C(T)	T	N	6
T	.88	A	C	N	4
W	.79	T	G	G	1
P	.74	C	C	N	4
S	.73	T(A)	C(G)	N	6
H	.67	C	A	Y	2
R	.53	C(A)	G	N	6
C	.52	T	G	Y	2

Synonymous codon bias

- In most organisms, the codons for an amino acid are not used with equal frequency
- For many organisms this may reflect differences in translational efficiency & accuracy
  - more highly expressed genes have stronger biases
- For mammals codon usage mainly reflects the GC content of the region in which the gene is found
  - GC content variation probably reflects GC-biased gene conversion

From *Initial sequencing and analysis of the human genome*, International Human Genome Sequencing Consortium, *Nature* 409, 860-921 (2001)

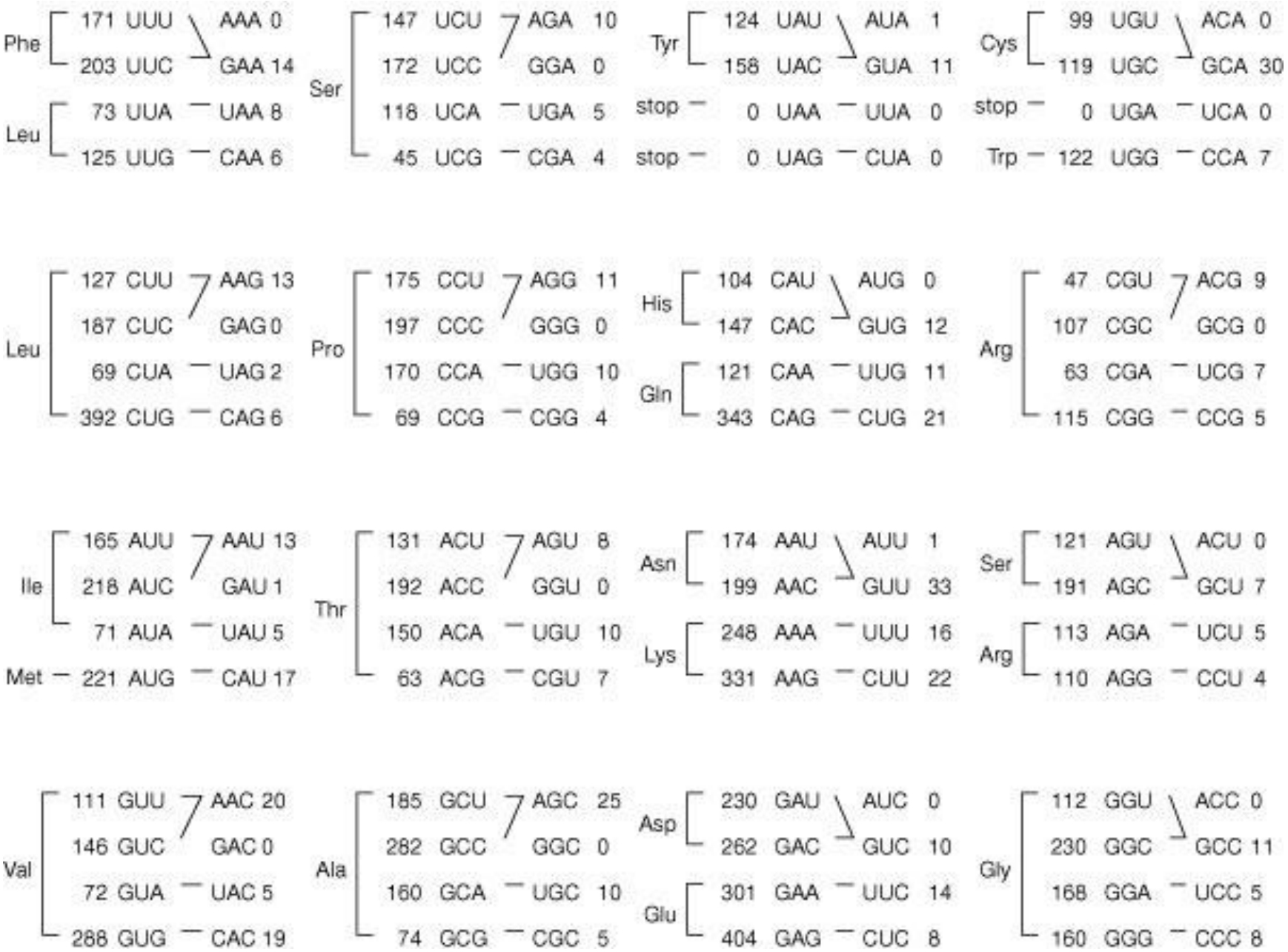


Figure 34 The human genetic code and associated tRNA genes. For each of the 64 codons, we show: the corresponding amino acid; the observed frequency of the codon per 10,000 codons; the codon; predicted wobble pairing to a tRNA anticodon (black lines); an unmodified tRNA anticodon sequence; and the number of tRNA genes found with this anticodon. For example, phenylalanine is encoded by UUU or UUC; UUC is seen more frequently, 203 to 171 occurrences per 10,000 total codons; both codons are expected to be decoded by a single tRNA anticodon type, GAA, using a G/U wobble; and there are 14 tRNA genes found with this anticodon. The modified anticodon sequence in the mature tRNA is not shown, even where post-transcriptional modifications can be confidently predicted (for example, when an A is used to decode a U/C third position, the A is almost certainly an inosine in the mature tRNA). The Figure also does not show the number of distinct tRNA species (such as distinct sequence families) for each anticodon; often there is more than one species for each anticodon.

Prokaryote HMMs

- Main types of sites:
  - Codon sites
  - Translation start sites (Shine-Dalgarno)
  - Promoter elements
    - Transcription factor binding sites
  - (RNA genes / RNA folding sites)
  - (replication origin)

- Simple 7-state prokaryote genome model:
  - 1 state for intergenic regions
  - 3 states for codon positions in top-strand genes
  - 3 for codon positions in bottom-strand genes

Amino Acid	Obs/Exp	1st codon base	2nd codon base	3rd codon base	# codons
E	1.92	G	A	R	2
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D	1.62	G	A	Y	2
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Q	1.22	C	A	R	2
I	1.16	A	T	Not G	3
A	1.14	G	C	N	4
G	1.05	G	G	N	4
V	.99	G	T	N	4
Y	.98	T	A	Y	2
L	.95	C(T)	T	N	6
T	.88	A	C	N	4
W	.79	T	G	G	1
P	.74	C	C	N	4
S	.73	T(A)	C(G)	N	6
H	.67	C	A	Y	2
R	.53	C(A)	G	N	6
C	.52	T	G	Y	2

Average codon biases (*lecture 3*)

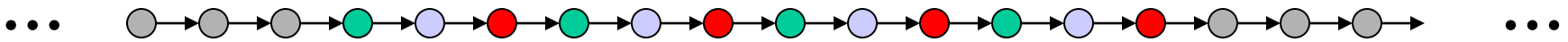
- At codon position 1,
  - purines (*A* and *G*) predominate among over-represented amino acids,
  - pyrimidines (*C* and *T*) among under-represented amino acids.
- At codon position 2,
  - *A* and *T* predominate among over-represented amino acids,
  - *C* and *G* among under-represented amino acids.
- Hypotheses to explain *RWR* codon preference:
  - (Neutralist) Vestige of ancestral code? (Shepherd)
  - (Selectionist) More efficiently translated?

- These biases are somewhat subtle – but strong enough to (often) distinguish
  - coding sequences (of reasonable length)

from

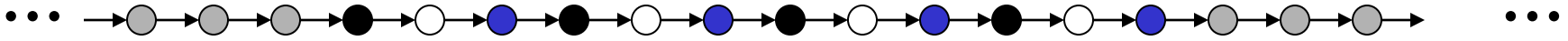
  - background sequence

7-state model for prokaryote genomes



- intergenic
- first codon position – top strand coding sequence
- second codon position – top strand coding sequence
- third codon position – top strand coding sequence
- first codon position – bottom strand coding sequence
- second codon position – bottom strand coding sequence
- third codon position – bottom strand coding sequence

a (very short!) ‘bottom-strand’ gene, in a different region of the genome:



- N.B. the emitted symbols are always ***top strand*** nucleotides!

A better HMM!

- Amino acid-specific codon blocks
  - Not really ‘sites’ as previously defined – may have more than one tRNA reader
  - Split the three 6-codon amino acids into 2 sites (4 + 2)
    - E.g. Leu: CTN and TTR ‘sites’
    - A single YTN site would also emit Phe codons
  - The other 17 aas are each 1 site
- ‘Start’ codon: NTG
  - Part of Shine-Dalgarno
- 2 Stop codon sites: TAR, TGA
- Total codon sites: $17 + 3 \times 2 + 1 + 2 = 26$

The Genetic Code

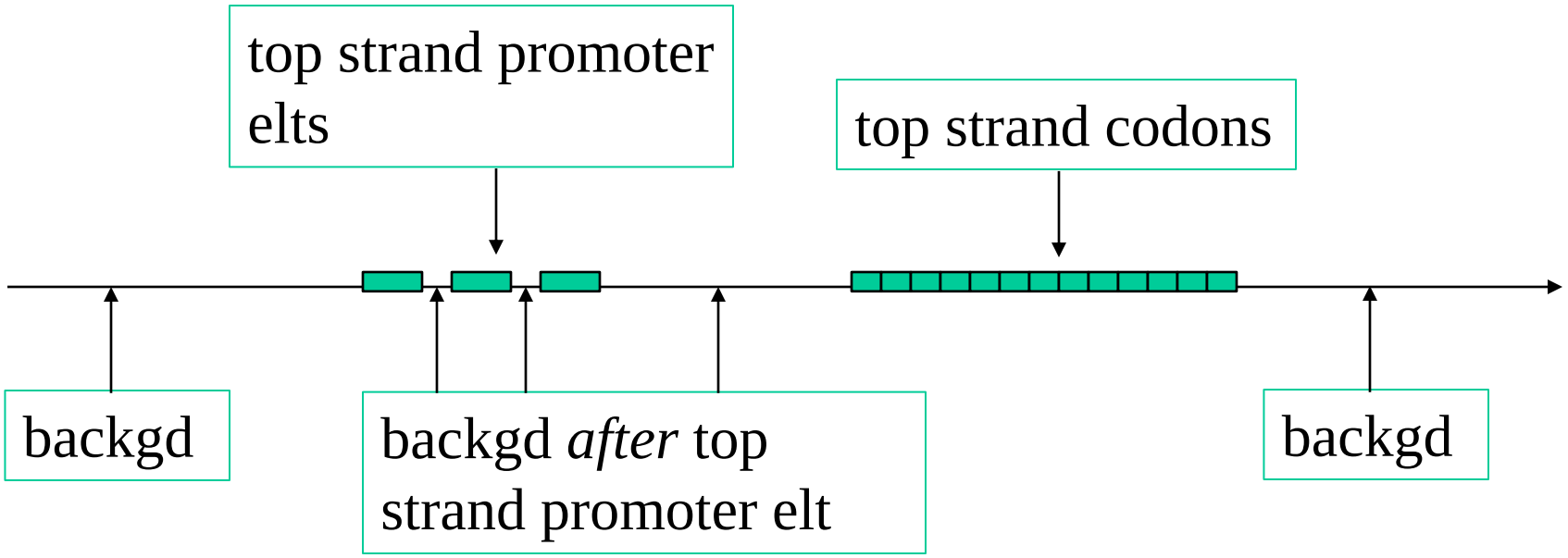
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

- Total codon ***states***

= 26 sites × 2 strands × 3 pos = 156

- Transitions within & between codons are the obvious ones
  - Unless one wishes to allow for frameshift sequencing errors!
- Also, states for promoter element sites
  - TF binding sites
- Ignore RNA genes
  - (identify by sequence similarity)
- Ignore replication origins
  - Often can identify after HMM analysis, by orientation biases

- Need more than one background state, to allow memory of where one is in a gene, and strand



- May need additional backgd states if promoter element *order* is important
- Role of ‘memory’ is to *reduce* impact of biologically implausible paths
  - Model may still work without these complications – but with reduced power
- Reasonable to constrain all backgd states to have *same emission probs*

- Use Viterbi or Baum-Welch training
  - (with appropriate top vs bottom constraints etc)

to find

  - Codon biases, aa freqs
  - Promoter elements
    - Include sites of size ~6, random initial emission probs
  - Shine-Dalgarno sequence preferences

Complications in Eukaryotes

- 5’ & 3’ splice sites
- poly A sites
- introns
  - Must retain memory of where codon is interrupted!
- 5’ & 3’UTR
- G+C variation

- Not difficult to set up an HMM with states corresponding to the above; ***but*** complex site spatial relationships are not well captured by Markov transition model:
  - Intron size constraints
  - Enhancers (possibly intronic!)
- Also:
  - alternative splicing
  - alternative promoters
  - overlapping sites

imply any single parse is incomplete