CSE 549: Models for ab initio Gene Finding

Genome of the Cow

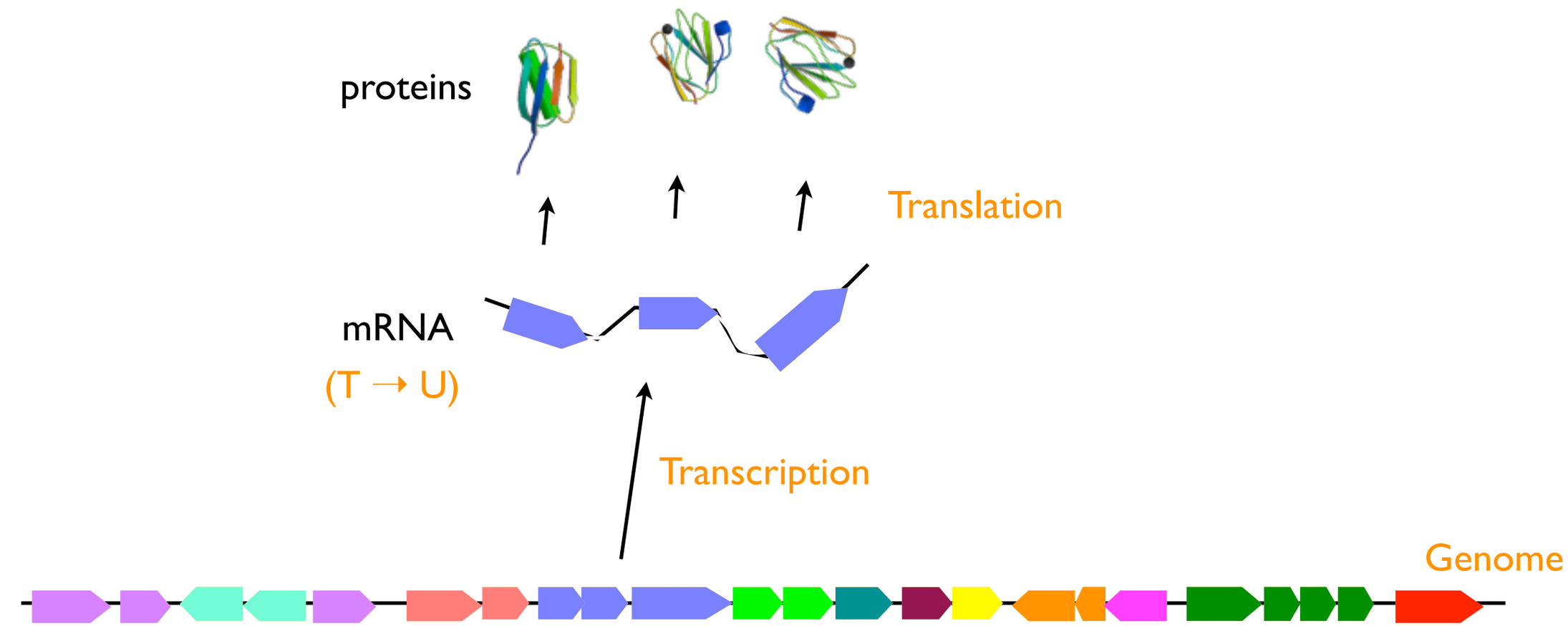
a sequence of 2.86 billion letters

enough letters to fill a million pages of a typical book.

```
TATGGAGCCAGGTGCCTGGGGCAACAAGACTGTGGTCACTGAATTCATCCTTCTTGGTCTAACAGAGAACATAG
AACTGCAATCCATCCTTTTTGCCATCTTCCTCTTTGCCTATGTGATCACAGTCGGGGGCAACTTGAGTATCCTG
GCCGCCATCTTTGTGGAGCCCAAACTCCACACCCCCATGTACTACTTCCTGGGGAACCTTTCTCTGCTGGACAT
TGGGTGCATCACTGTCACCATTCCTCCCATGCTGGCCTGTCTCCTGACCCACCAATGCCGGGTTCCCTATGCAG
CCTGCATCTCACAGCTCTTCTTTTTCCACCTCCTGGCTGGAGTGGACTGTCACCTCCTGACAGCCATGGCCTAC
GACCGCTACCTGGCCATTTGCCAGCCCCTCACCTATAGCATCCGCATGAGCCGTGACGTCCAGGGAGCCCTGGT
GGCCGTCTGCTGCTCCATCTCCTTCATCAATGCTCTGACCCACACAGTGGCTGTGTCTGTGCTGGACTTCTGCG
GCCCTAACGTGGTCAACCACTTCTACTGTGACCTCCCGCCCCTTTTCCAGCTCTCCTGCTCCAGCATCCACCTC
AACGGGCAGCTACTTTTCGTGGGGGCCACCTTCATGGGGGTGGTCCCCATGGTCTTCATCTCGGTATCCTATGC
CCACGTGGCAGCCGCAGTCCTGCGGATCCGCTCGGCAGAGGGCAGGAAGAAAGCCTTCTCCACGTGTGGCTCCC
ACCTCACCGTGGTCTGCATCTTTTATGGAACCGGCTTCTTCAGCTACATGCGCCTGGGCTCCGTGTCCGCCTCA
GACAAGGACAAGGGCATTGGCATCCTCAACACTGTCATCAGCCCCATGCTGAACCCACTCATCTACAGCCTCCG
GAACCCTGATGTGCAGGGCGCCCTGAAGAGGTTGCTGACAGGGAAGCGGCCCCCGGAGTG ...
```

* slide from Carl Kingsford

"Central Dogma" of Biology



DNA =

- double-stranded, linear molecule
- each strand is string over {A,C,G,T}
- strands are complements of each other (A ↔ T; C ↔ G)
- substrings encode for genes  most of which encode for proteins

* slide from Carl Kingsford

The Genetic Code

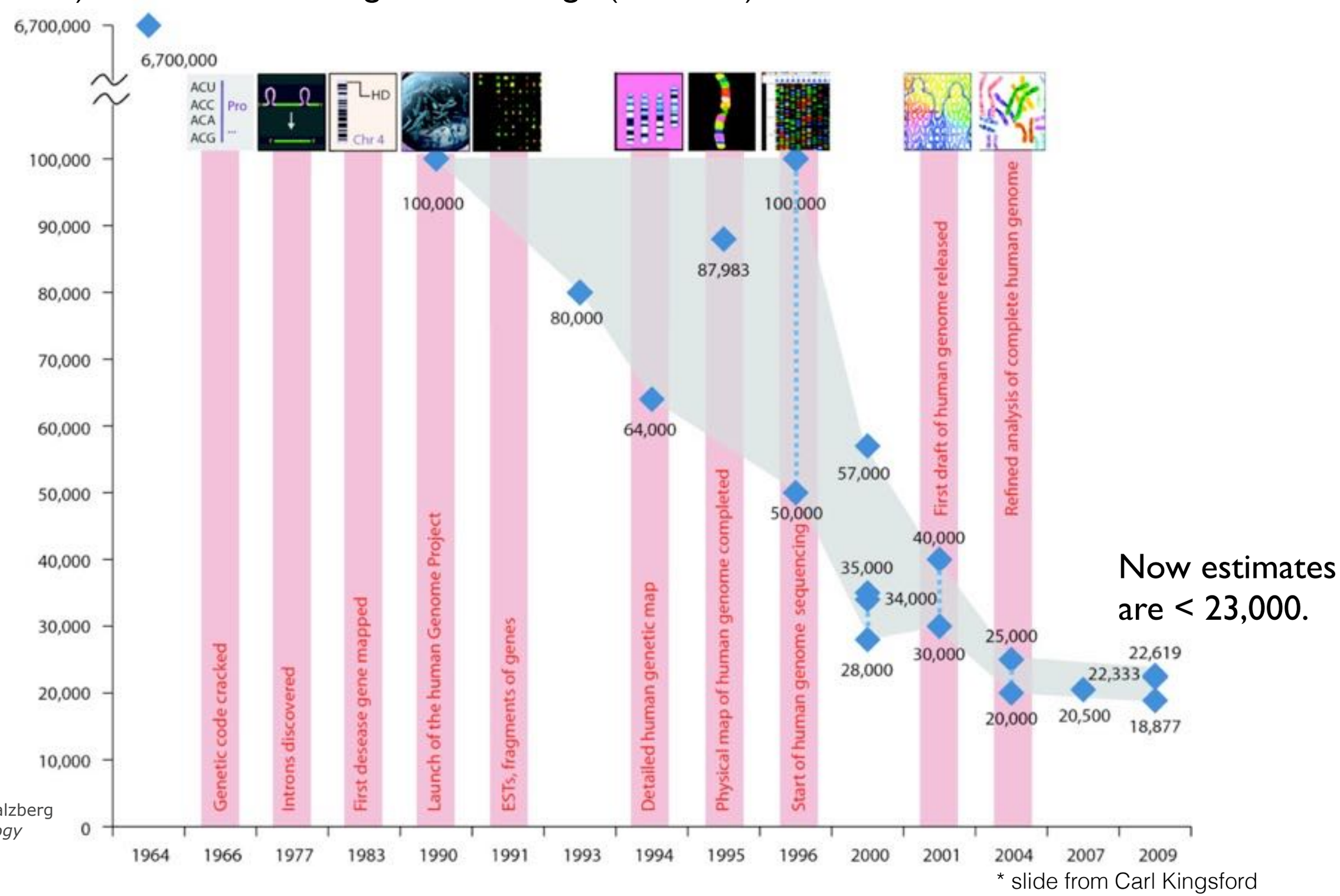
1st base	2nd base: U		2nd base: C		2nd base: A		2nd base: G	
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine	UAU	(Tyr/Y) Tyrosine	UGU	(Cys/C) Cysteine
	UUC	(Phe/F) Phenylalanine	UCC	(Ser/S) Serine	UAC	(Tyr/Y) Tyrosine	UGC	(Cys/C) Cysteine
	UUA	(Leu/L) Leucine	UCA	(Ser/S) Serine	UAA	Ochre *Stop*	UGA	Opal *Stop*
	UUG	(Leu/L) Leucine	UCG	(Ser/S) Serine	UAG	Amber *Stop*	UGG	(Trp/W) Tryptophan
C	CUU	(Leu/L) Leucine	CCU	(Pro/P) Proline	CAU	(His/H) Histidine	CGU	(Arg/R) Arginine
	CUC	(Leu/L) Leucine	CCC	(Pro/P) Proline	CAC	(His/H) Histidine	CGC	(Arg/R) Arginine
	CUA	(Leu/L) Leucine	CCA	(Pro/P) Proline	CAA	(Gln/Q) Glutamine	CGA	(Arg/R) Arginine
	CUG	(Leu/L) Leucine	CCG	(Pro/P) Proline	CAG	(Gln/Q) Glutamine	CGG	(Arg/R) Arginine
A	AUU	(Ile/I) Isoleucine	ACU	(Thr/T) Threonine	AAU	(Asn/N) Asparagine	AGU	(Ser/S) Serine
	AUC	(Ile/I) Isoleucine	ACC	(Thr/T) Threonine	AAC	(Asn/N) Asparagine	AGC	(Ser/S) Serine
	AUA	(Ile/I) Isoleucine	ACA	(Thr/T) Threonine	AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine
	AUG [A]	(Met/M) Methionine	ACG	(Thr/T) Threonine	AAG	(Lys/K) Lysine	AGG	(Arg/R) Arginine
G	GUU	(Val/V) Valine	GCU	(Ala/A) Alanine	GAU	(Asp/D) Aspartic acid	GGU	(Gly/G) Glycine
	GUC	(Val/V) Valine	GCC	(Ala/A) Alanine	GAC	(Asp/D) Aspartic acid	GGC	(Gly/G) Glycine
	GUA	(Val/V) Valine	GCA	(Ala/A) Alanine	GAA	(Glu/E) Glutamic acid	GGA	(Gly/G) Glycine
	GUG	(Val/V) Valine	GCG	(Ala/A) Alanine	GAG	(Glu/E) Glutamic acid	GGG	(Gly/G) Glycine

- There are 20 different amino acids & 64 different codons.
- Lots of different ways to encode for each amino acid.
- The 3rd base is typically less important for determining the amino acid
- Three different "stop" codons that signal the end of the gene
- Start codons differ depending on the organisms, but AUG is often used.

* slide from Carl Kingsford

Estimates for the # of Human Genes

Before human genome sequence was available, many
(but not all) estimates for # of genes were high (> 80,000).

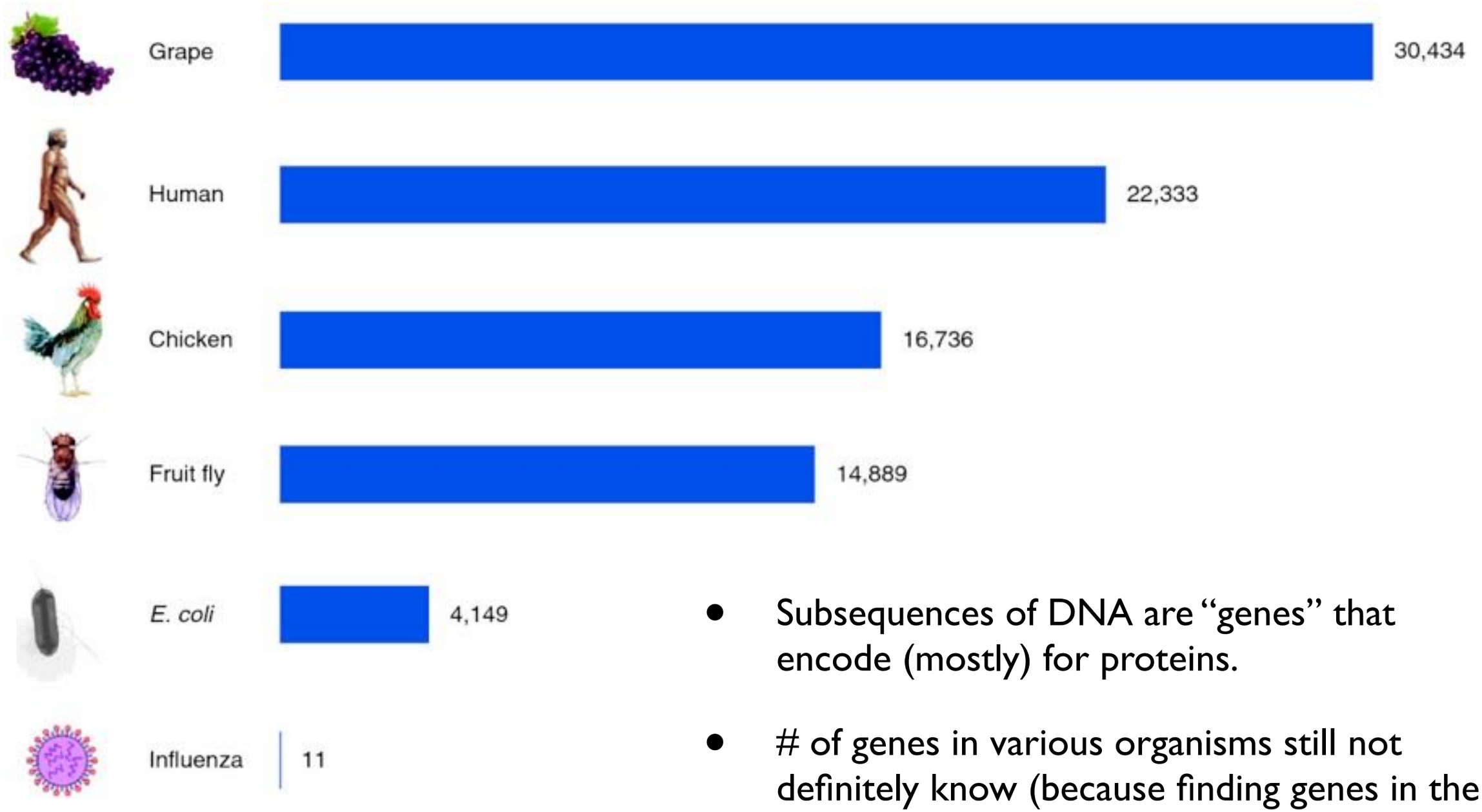


Now estimates are < 23,000.

Pertea and Salzberg
Genome Biology
2010 **11**:206

* slide from Carl Kingsford

of Genes in Various Organisms



Pertea and Salzberg
Genome Biology 2010
11:206

- Subsequences of DNA are "genes" that encode (mostly) for proteins.
- # of genes in various organisms still not definitely know (because finding genes in the sequence is a hard problem that we will talk about).
- But there are reasonably good estimates.

* slide from Carl Kingsford

Finding Genes

We'll break gene finding methods into 3 main categories.

ab initio

latin — "from the beginning"

w/o experimental evidence

based on predictive modeling

how well do genomic sequences score under our "gene model"?

comparative

make use of knowledge across species

a known human gene is strong evidence for a chimp gene

many "housekeeping" genes are incredibly similar across highly divergent species

combined / extrinsic

Make use of experimental evidence (e.g. RNA-seq)

Evidence highlights transcribed regions

Gene structure extracted from evidence (potentially combined with model predictions)

Ab Initio Prediction

Today, we'll focus mainly on *ab initio* prediction, but will touch on comparative prediction.

- How do we build models of genes?

- How do we use these models to predict genes?

- What features must the models capture?

- Where are the models good, where do they fail?

Prokaryotic Gene Prediction

Genes in prokaryotes *generally* have a simple structure

gene

start codon

stop codon

nonpolar polar basic acidic (stop codon)

Standard genetic code

1st base	2nd base U		2nd base C		2nd base A		2nd base G		3rd base
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine	UAU	(Tyr/Y) Tyrosine	UGU	(Cys/C) Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	(Leu/L) Leucine	UCA		UAA	Stop (*Ochre*)	UGA	Stop (*Opal*)	A
	UUG		UCG		UAG	Stop (*Amber*)	UGG	(Trp/W) Tryptophan	G
C	CUU		CCU	(Pro/P) Proline	CAU	(His/H) Histidine	CGU	(Arg/R) Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	(Ile/I) Isoleucine	ACU	(Thr/T) Threonine	AAU	(Asn/N) Asparagine	AGU	(Ser/S) Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	AUG[W]	(Met/M) Methionine	ACG		AAG		AGG		G
G	GUU	(Val/V) Valine	GCU	(Ala/A) Alanine	GAU	(Asp/D) Aspartic acid	GGU	(Gly/G) Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GUG		GCG		GAG		GGG		G

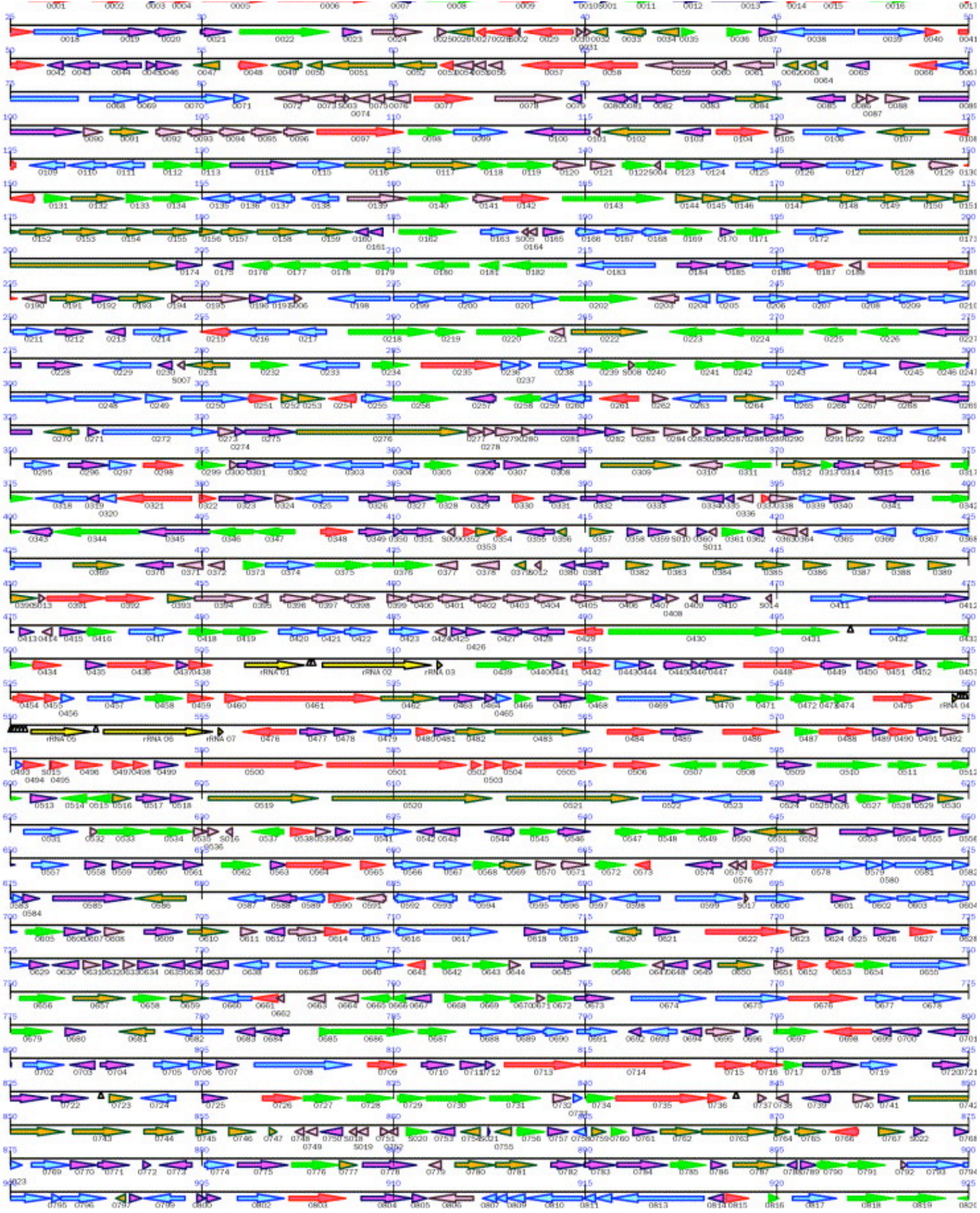
http://en.wikipedia.org/wiki/Genetic_code

Bacterial genes

Genes (colored arrows) packed tightly into the *Staphylococcus aureus* genome

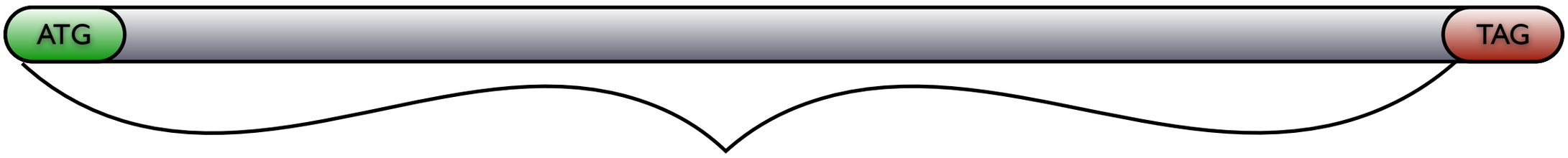
In bacteria, one gene corresponds to one continuous interval on the genome

We have good methods for predicting where these genes are



* slide from Ben Langmead

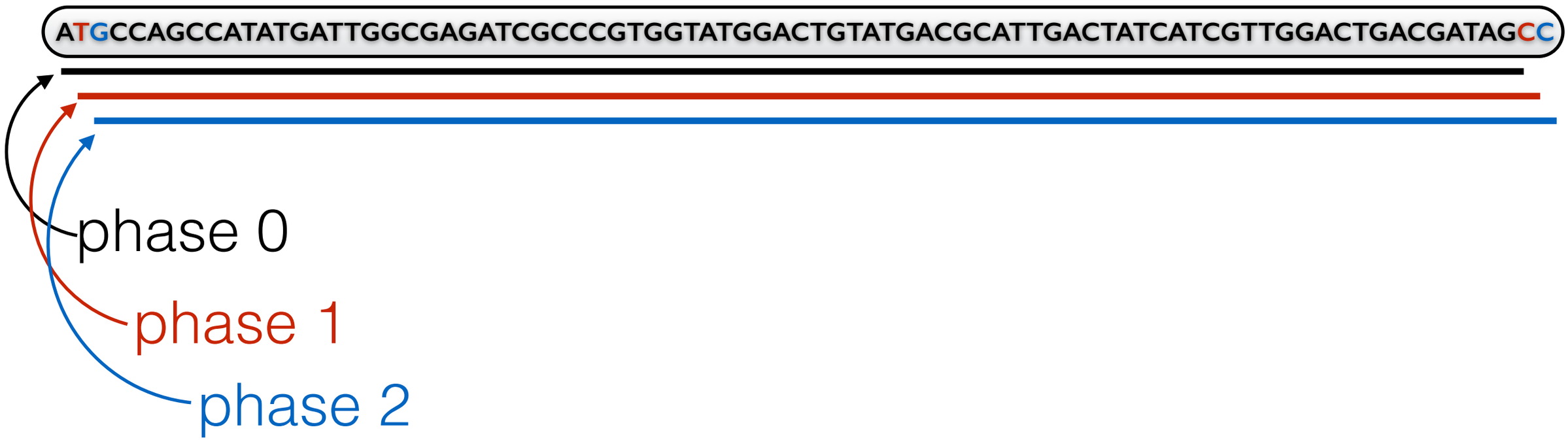
Prokaryotic Gene Prediction



We call the region from a start codon until a stop codon in the same "phase" an **open reading frame** (ORF).

ORFs are possible genes. All genes are ORFs, but all ORFs are **not genes**.

Prokaryotic Gene Prediction



The codons are read off as consecutive, disjoint sequences of 3 bases each. But we don't know where the first codon of a gene starts.

Each strand of DNA contains 3 "reading frames". Consecutive, disjoint, codons in a gene are in the same "phase" (same index mod 3) as their predecessors.

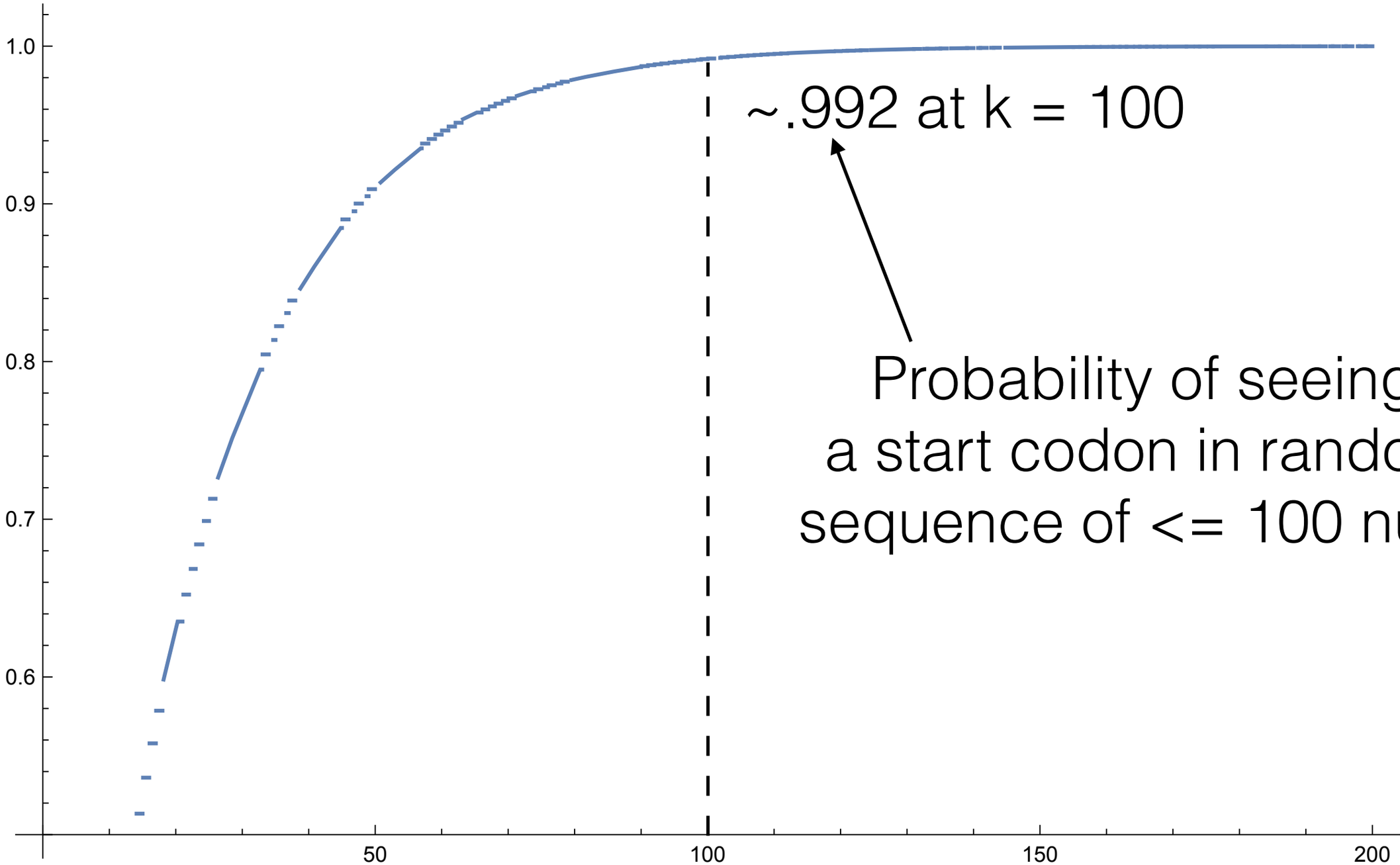
Prokaryotic Gene Prediction

There's 1 start codon and 3 stop codons. In a random sequence, the probability of seeing 3 bases that represent a start codon is 1/64. The prob. of seeing 3 bases that represent a stop codon is 3/64.

Probability of *not observing* a stop codon in a random sequence of length L *decays* geometrically in L.

Prokaryotic Gene Prediction

CDF(Geom(3/64)) = 1-(1-(3/64))^k



Prokaryotic Gene Prediction

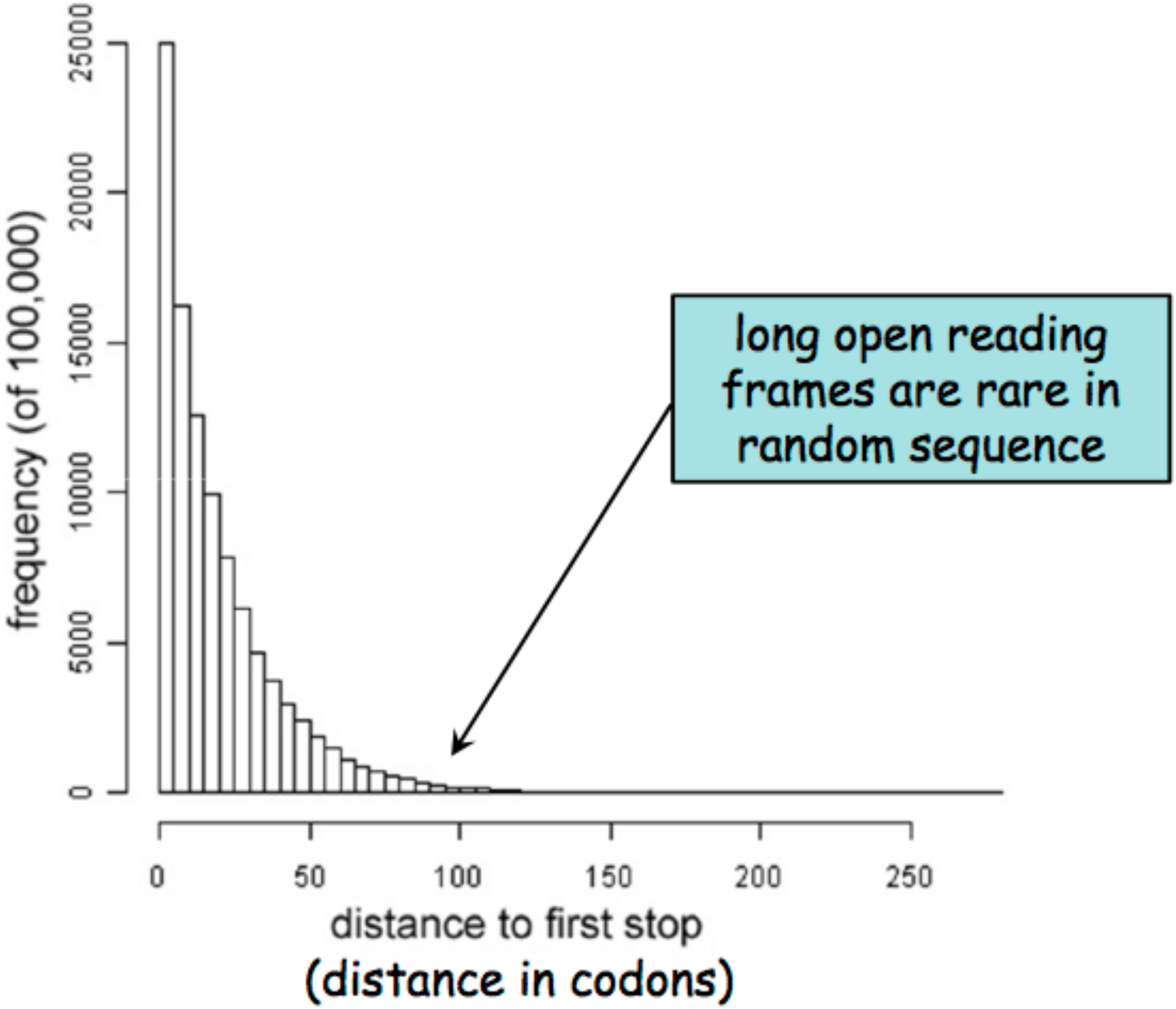


Image from: http://elbo.gs.washington.edu/courses/GS_559_11_wi/slides/17A_GenePrediction.pdf

Gene Prediction

So, even finding a long, non-interrupted ORF can be strong evidence that a stretch of sequence is a gene.

This leads to a **very simple** idea for a prokaryotic gene finding algorithm.

http://en.wikipedia.org/wiki/Codon_usage_bias

A Simple Gene Finder

1. Find all stop codons in genome

2. For each stop codon, find the in-frame start codon farthest upstream of the stop codon, without crossing another in-frame stop codon.

GGC **TAG** **ATG** AGG GCT CTA ACT **ATG** GGC GCG **TAA**

Each substring between the start and stop codons is called an ORF
"open reading frame"

3. Return the "long" ORF as predicted genes.

3 out of the 64 possible codons are stop codons ⇒ in random DNA, every 22nd codon is expected to be a stop.

Gene Prediction

Another type of evidence is codon usage bias — a difference in the frequency of occurrence of synonymous codons in genic DNA.

http://en.wikipedia.org/wiki/Codon_usage_bias

Gene Prediction

Codon usage bias in anthrax

Bacillus anthracis str. A2012 **[gbbct]: 308 CDS's (64833 codons)**

fields: [triplet] [frequency: **per thousand**] ([number])

UUU	34.7 (2249)	UCU	15.7 (1015)	UAU	34.5 (2238)	UGU	6.8 (441)
UUC	11.7 (761)	UCC	4.5 (293)	UAC	10.4 (672)	UGC	2.6 (167)
UUA	41.8 (2709)	UCA	14.2 (921)	UAA	2.8 (184)	UGA	1.0 (65)
UUG	12.9 (835)	UCG	3.9 (256)	UAG	0.9 (59)	UGG	10.2 (661)
CUU	14.5 (937)	CCU	10.2 (662)	CAU	16.7 (1084)	CGU	11.0 (715)
CUC	3.6 (233)	CCC	2.5 (165)	CAC	4.7 (305)	CGC	2.9 (188)
CUA	13.7 (888)	CCA	12.6 (817)	CAA	30.7 (1993)	CGA	6.6 (425)
CUG	5.0 (326)	CCG	4.5 (292)	CAG	10.3 (671)	CGG	1.8 (116)
AUU	43.5 (2817)	ACU	13.7 (886)	AAU	44.1 (2856)	AGU	16.7 (1082)
AUC	11.7 (758)	ACC	5.1 (328)	AAC	14.4 (932)	AGC	6.2 (403)
AUA	24.9 (1616)	ACA	25.9 (1678)	AAA	66.8 (4332)	AGA	14.6 (948)
AUG	25.3 (1642)	ACG	8.6 (555)	AAG	25.8 (1671)	AGG	4.5 (294)
GUU	19.5 (1263)	GCU	17.0 (1105)	GAU	38.9 (2522)	GGU	17.6 (1142)
GUC	5.4 (347)	GCC	4.4 (285)	GAC	9.1 (589)	GGC	5.8 (379)
GUA	26.1 (1694)	GCA	22.0 (1429)	GAA	55.1 (3570)	GGA	19.7 (1276)
GUG	11.0 (711)	GCG	7.6 (490)	GAG	19.7 (1276)	GGG	9.5 (614)

Coding GC 33.87% 1st letter GC 43.97% 2nd letter GC 30.99% 3rd letter GC 26.64%

Gene Finding as a Machine Learning Problem

- Given training examples of some known genes, can we distinguish ORFs that are genes from those that are not?

- **Idea**: can use distribution of codons to find genes.
  - every codon should be about equally likely in non-gene DNA.
  - every organism has a slightly different bias about how often certain codons are preferred.
  - could also use frequencies of longer strings (k-mers).

* slide from Carl Kingsford

An Improved Simple Gene Finder

- Score each ORF using the product of the probability of each codon:

$$GFScore(g) = Pr(codon_1) \times Pr(codon_2) \times Pr(codon_3) \times \ldots \times Pr(codon_n)$$

$$\log[GFScore(g)] = \log[Pr(codon_1)] + \log[Pr(codon_2)] + \ldots + \log[Pr(codon_n)]$$

But: as genes get longer, GFScore(g) will decrease.

One option: also score the sequence under a null / non-gene model NScore(g).

We can use the log-odds ratio of GFScore(g) to Score (G) to predict wether or not this ORF is a gene.

* slide from Carl Kingsford

Glimmer

Salzberg et al., NAR, 1998

- Score ORFs using 6 Markov Models (not hidden):
  - 1 model for each reading frame (3 forward, 3 reverse)
- ORFs for which the highest scoring reading frame exceed some threshold are output as "putative" genes.

- Use "Interpolated Markov models" to adapt to data availability
- Handle overlapping ORFs

* slide from Carl Kingsford

Interpolated HMMs

Length of the sequence

Sequence

String ending at position x

$$P(S|M) = \sum_{x=1}^{n} \mathbf{IMM}_8(S_x)$$

Model

$$P_i(S_x) = P(s_x|S_{x,i}) = \frac{f(S_{x,i})}{\sum_{b\in\{acgt\}} f(S_{x,i}, b)}$$

Sequence ending at pos x of length i — length i "context"

IMM score is a linear combination of 8th, 7th, …, 0th order models:

Weight of the k^{th}-order context ending at position x-1

$$\mathbf{IMM}_k(S_x) = \lambda_k(S_{x-1}) \bullet P_k(S_x) + [1 - \lambda_k(S_{x-1})] \bullet \mathbf{IMM}_{k-1}(S_x)$$

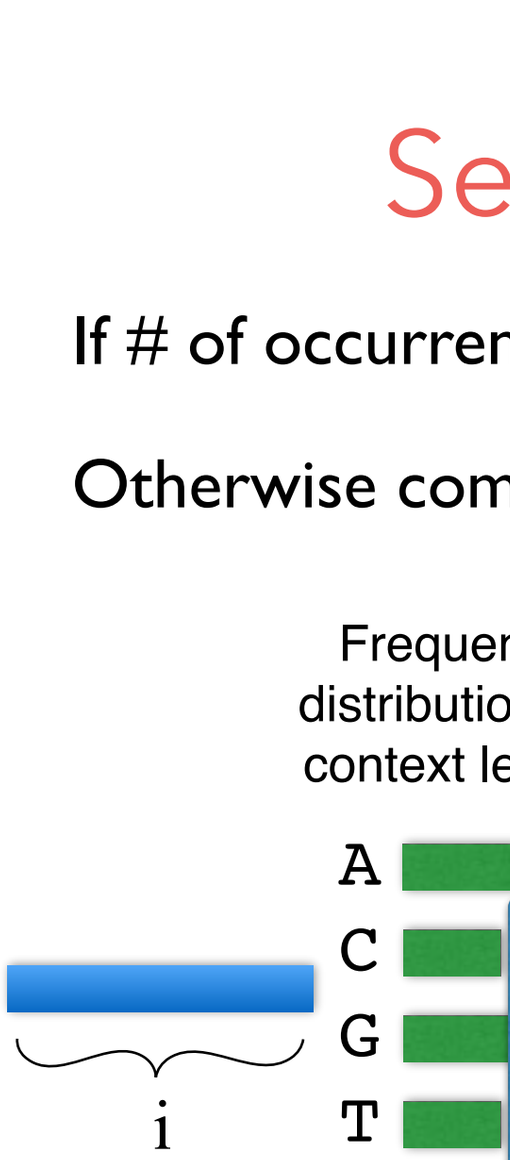
Probability of letter at position x from a *k*th-order model

* slide from Carl Kingsford

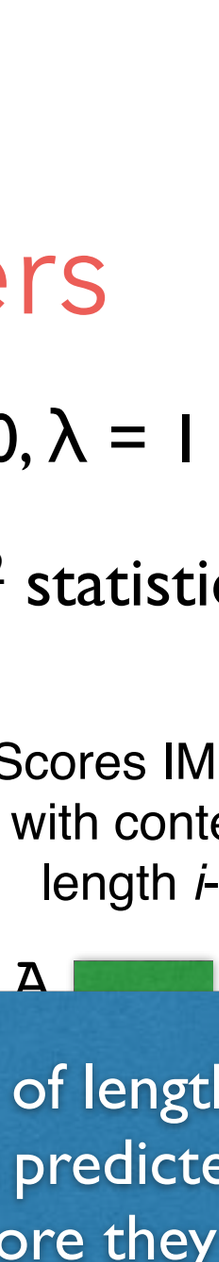
Setting Parameters

- If # of occurrences of context k-mer ≥ 400, λ = 1
- Otherwise compare the following with a χ^2 statistic:

Frequency f distribution with context length i



Scores IMM_{i-1} with context length *i*-1



When c is large, distribution of length *i* frequencies differs from that predicted by the i-1 order IMM. The more they differ, the more we weight them.

- Set λ as follows, where come from the IMM distribution:

$$\lambda_i(S_{x-1}) = \begin{cases} 0.0 & \text{if } c < 0.50 \\ \frac{c}{400} \sum_{b \in \{acgt\}} f(s_1 s_2 \ldots s_i b) & \text{if } c \geq 0.50 \end{cases}$$

* slide from Carl Kingsford

IMM vs. 5th Order Markov Model

Model	Genes found	Genes missed	Additional genes
GLIMMER IMM	1680 (97.8%)	37	209
5th-Order Markov	1574 (91.7%)	143	104

The first column indicates how many of the 1717 annotated genes in *H.influenzae* were found by each algorithm. The 'additional genes' column shows how many extra genes, not included in the 1717 annotated entries, were called genes by each method.

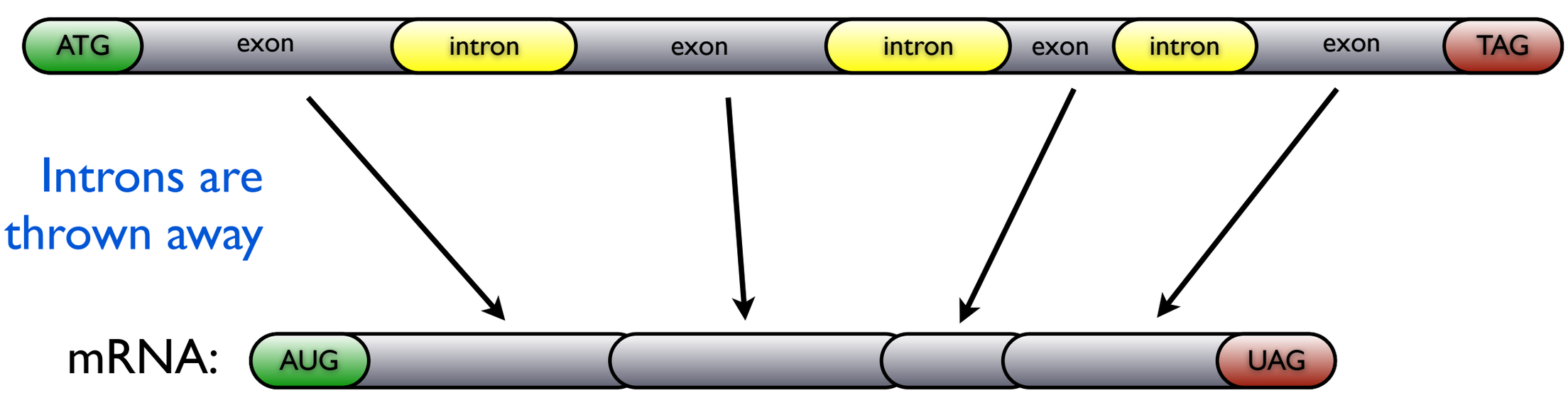
Salzberg et al., NAR, 1998

* slide from Carl Kingsford

Eukaryotic Genes & Exon Splicing

Prokaryotic (bacterial) genes look like this:

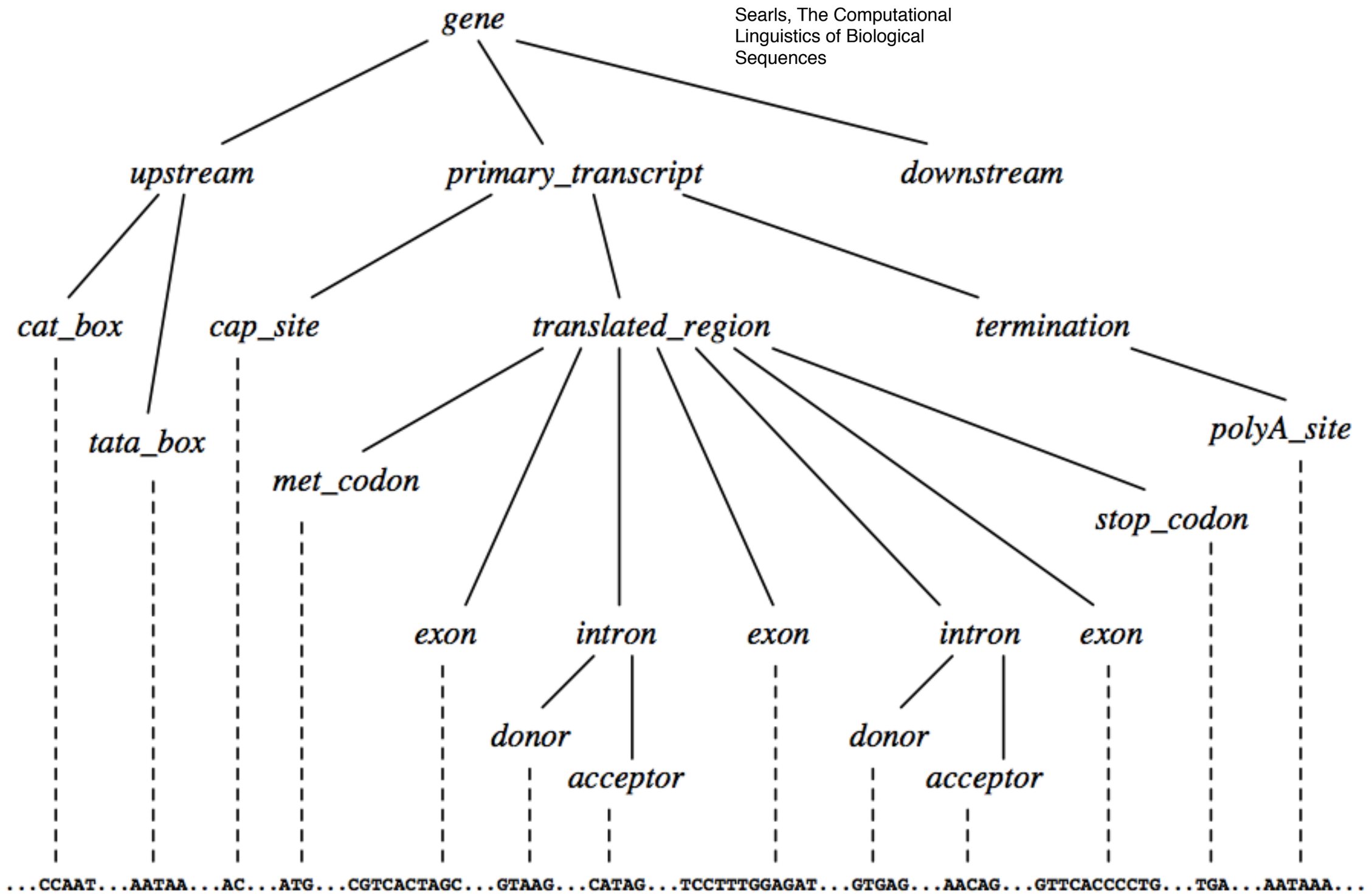
Eukaryotic genes usually look like this:



This spliced RNA is what is translated into a protein.

* slide from Carl Kingsford

Hypothetical Eukaryotic Gene Parse Tree



Searls, The Computational Linguistics of Biological Sequences

* slide from Carl Kingsford

A human gene

chr11:5246500-5248500 (reverse strand):

ATATCTTAGAGGGAGGGCTGAGGGTTTGAAGTCCAACTCCTAAGCCAGTGCCAGAAGAGCCAAGGACAGGTACGGCTGTC
ATCACTTAGACCTCACCCTGTGGAGCCACACCCTAGGGTTGGCCAATCTACTCCCAGGAGCAGGGAGGGCAGGAGCCAGG
GCTGGGCATAAAAGTCAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAA
CAGACACCATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTT
GGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGA
CAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATTGGTCTATTTTCCCACCCTTAGGCTGCTGGTG
GTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGT
GAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCA
CACTGAGTGAGCTGCACTGTGACAAGCTGCACGTGGATCCTGAGAACTTCAGGGTGAGTCTATGGGACGCTTGATGTTTT
CTTTCCCCTTCTTTTCTATGGTTAAGTTCATGTCATAGGAAGGGGATAAGTAACAGGGTACAGTTTAGAATGGGAAACAG
ACGAATGATTGCATCAGTGTGGAAGTCTCAGGATCGTTTTAGTTTCTTTTATTTGCTGTTCATAACAATTGTTTTCTTTT
GTTTAATTCTTGCTTTCTTTTTTTTTCTTCTCCGCAATTTTTACTATTATACTTAATGCCTTAACATTGTGTATAACAAA
AGGAAATATCTCTGAGATACATTAAGTAACTTAAAAAAAAACTTTACACAGTCTGCCTAGTACATTACTATTTGGAATAT
ATGTGTGCTTATTTGCATATTCATAATCTCCCTACTTTATTTTCTTTTATTTTTAATTGATACATAATCATTATACATAT
TTATGGGTTAAAGTGTAATGTTTTAATATGTGTACACATATTGACCAAATCAGGGTAATTTTGCATTTGTAATTTTAAAA
AATGCTTTCTTCTTTTAATATACTTTTTTGTTTATCTTATTTCTAATACTTTCCCTAATCTCTTTCTTTCAGGGCAATAA
TGATACAATGTATCATGCCTCTTTGCACCATTCTAAAGAATAACAGTGATAATTTCTGGGTTAAGGCAATAGCAATATCT
CTGCATATAAATATTTCTGCATATAAATTGTAACTGATGTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCCAGCTA
CCATTCTGCTTTTATTTTATGGTTGGGATAAGGCTGGATTATTCTGAGTCCAAGCTAGGCCCTTTTGCTAATCATGTTCA
TACCTCTTATCTTCCTCCCACAGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCACTTTGGCAAAGAATTCACC
CCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCTGGTGTGGCTAATGCCCTGGCCCACAAGTATCACTAAGCTCGCTT
TCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTT
GAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAATGATGTATTTAAATTATTTCTGAATATTTTACTA
AAAAGGGAATGTGGGAGGTCAGTGCATTTAAAACATAAAGAAATGAAGAGCTAGTTCAAACCTTGGGAAAATACACTATA
TCTTAAACTCCATGAAAGAAGGTGAGGCTGCAAACAGCTAATGCACATTGGCAACAGCCCCTGATGCATATGCCTTATTC

* slide from Ben Langmead

A human gene

chr11:5246500-5248500 (reverse strand):

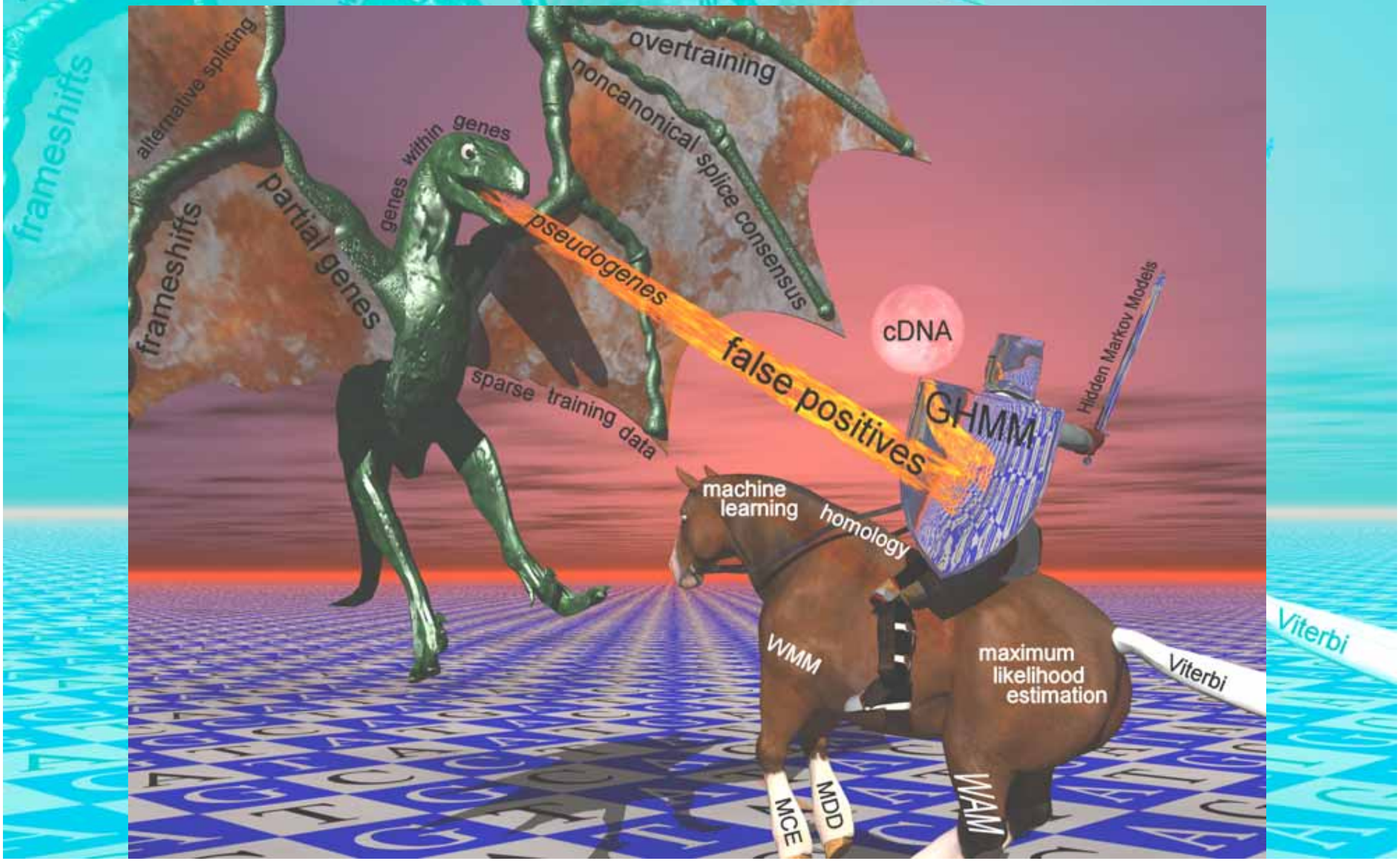
ATATCTTAGAGGGAGGGCTGAGGGTTTGAAGTCCAACTCCTAAGCCAGTGCCAGAAGAGCCAAGGACAGGTACGGCTGTC
ATCACTTAGACCTCACCCTGTGGAGCCACACCCTAGGGTTGGCCAATCTACTCCCAGGAGCAGGGAGGGCAGGAGCCAGG
GCTGGGCATAAAAGTCAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAACTGTGTTCACTAGCAACCTCAAA
CAGACACCATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTT
GGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCATGTGGAGA
CAGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCCTATTGGTCTATTTTCCCACCCTTAGGCTGCTGGTG
GTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAACCCTAAGGT
GAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCA
CACTGAGTGAGCTGCACTGTGACAAGCTGCACGTGGATCCTGAGAACTTCAGGGTGAGTCTATGGGACGCTTGATGTTTT
CTTTCCCCTTCTTTTCTATGGTTAAGTTCATGTCATAGGAAGGGGATAAGTAACAGGGTACAGTTTAGAATGGGAAACAG
ACGAATGATTGCATCAGTGTGGAAGTCTCAGGATCGTTTTAGTTTCTTTTATTTGCTGTTCATAACAATTGTTTTCTTTT
GTTTAATTCTTGCTTTCTTTTTTTTCTTCTCCGCAATTTTTACTATTATACTTAATGCCTTAACATTGTGTATAACAAA
AGGAAATATCTCTGAGATACATTAAGTAACTTAAAAAAAAACTTTACACAGTCTGCCTAGTACATTACTATTTGGAATAT
ATGTGTGCTTATTTGCATA[illegible]TACATAATCATTATACATAT
TTATGGGTTAAAGTGTAA[illegible]TTGCATTTGTAATTTTAAAA
AATGCTTTCTTCTTTTAATATACTTTTTTGTTTATCTTATTTCTAATACTTTCCCTAATCTCTTTCTTTCAGGGCAATAA
TGATACAATGTATCATGCCTCTTTGCACCATTCTAAAGAATAACAGTGATAATTTCTGGGTTAAGGCAATAGCAATATCT
CTGCATATAAATATTTCTGCATATAAATTGTAACTGATGTAAGAGGTTTCATATTGCTAATAGCAGCTACAATCCAGCTA
CCATTCTGCTTTTATTTTATGGTTGGGATAAGGCTGGATTATTCTGAGTCCAAGCTAGGCCCTTTTGCTAATCATGTTCA
TACCTCTTATCTTCCTCCCACAGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCCCATCACTTTGGCAAAGAATTCACC
CCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGGCTGGTGTGGCTAATGCCCTGGCCCACAAGTATCACTAAGCTCGCTT
TCTTGCTGTCCAATTTCTATTAAAGGTTCCTTTGTTCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTT
GAGCATCTGGATTCTGCCTAATAAAAAACATTTATTTTCATTGCAATGATGTATTTAAATTATTTCTGAATATTTTACTA
AAAAGGGAATGTGGGAGGTCAGTGCATTTAAAACATAAAGAAATGAAGAGCTAGTTCAAACCTTGGGAAAATACACTATA
TCTTAAACTCCATGAAAGAAGGTGAGGCTGCAAACAGCTAATGCACATTGGCAACAGCCCCTGATGCATATGCCTTATTC

Homo sapiens hemoglobin, beta (HBB)

* slide from Ben Langmead

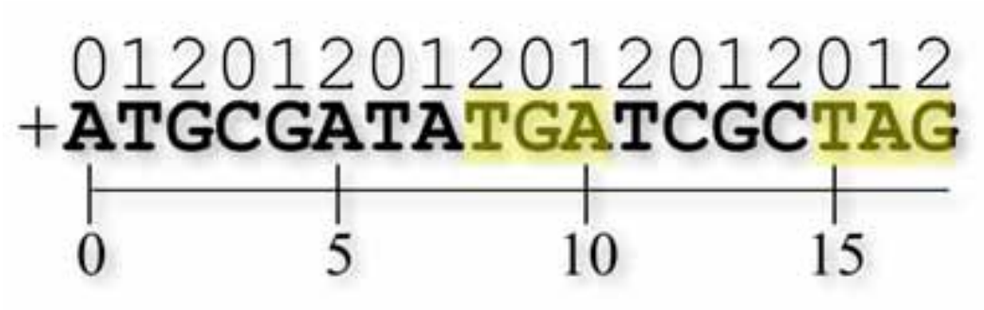
Advancing the State-of-the-art in
Computational Gene Prediction

Bill Majoros (bmajoros@duke.edu)

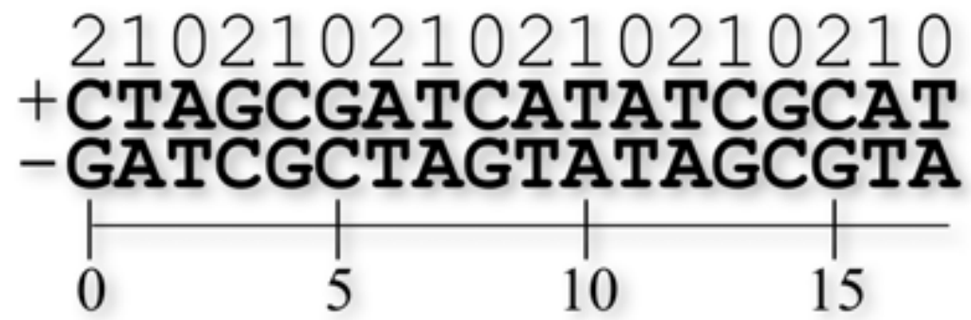


Following slides (marked #) are from: http://www.geneprediction.org/tutorial-majoros.pdf

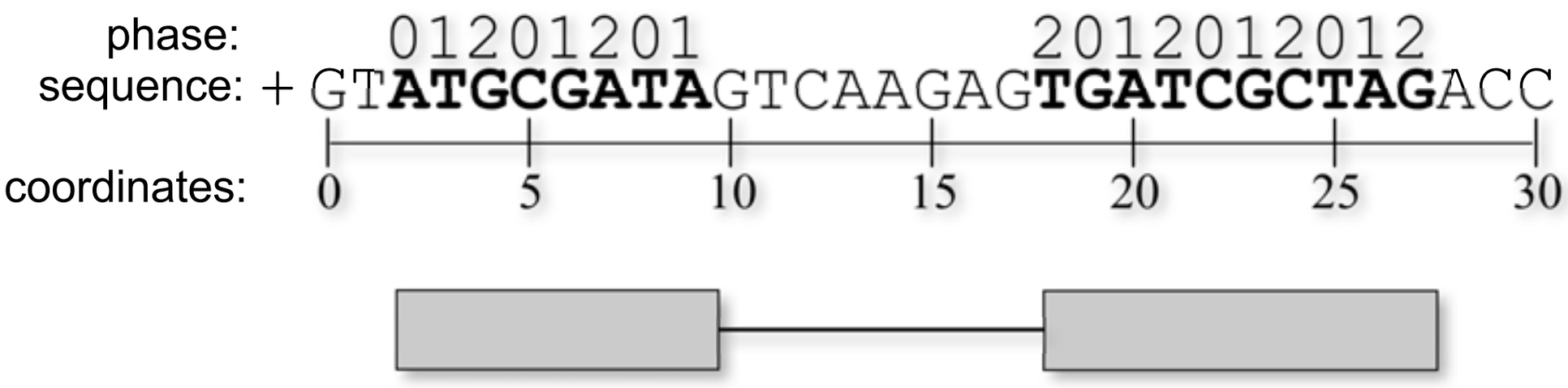
Phase Constraints



forward strand

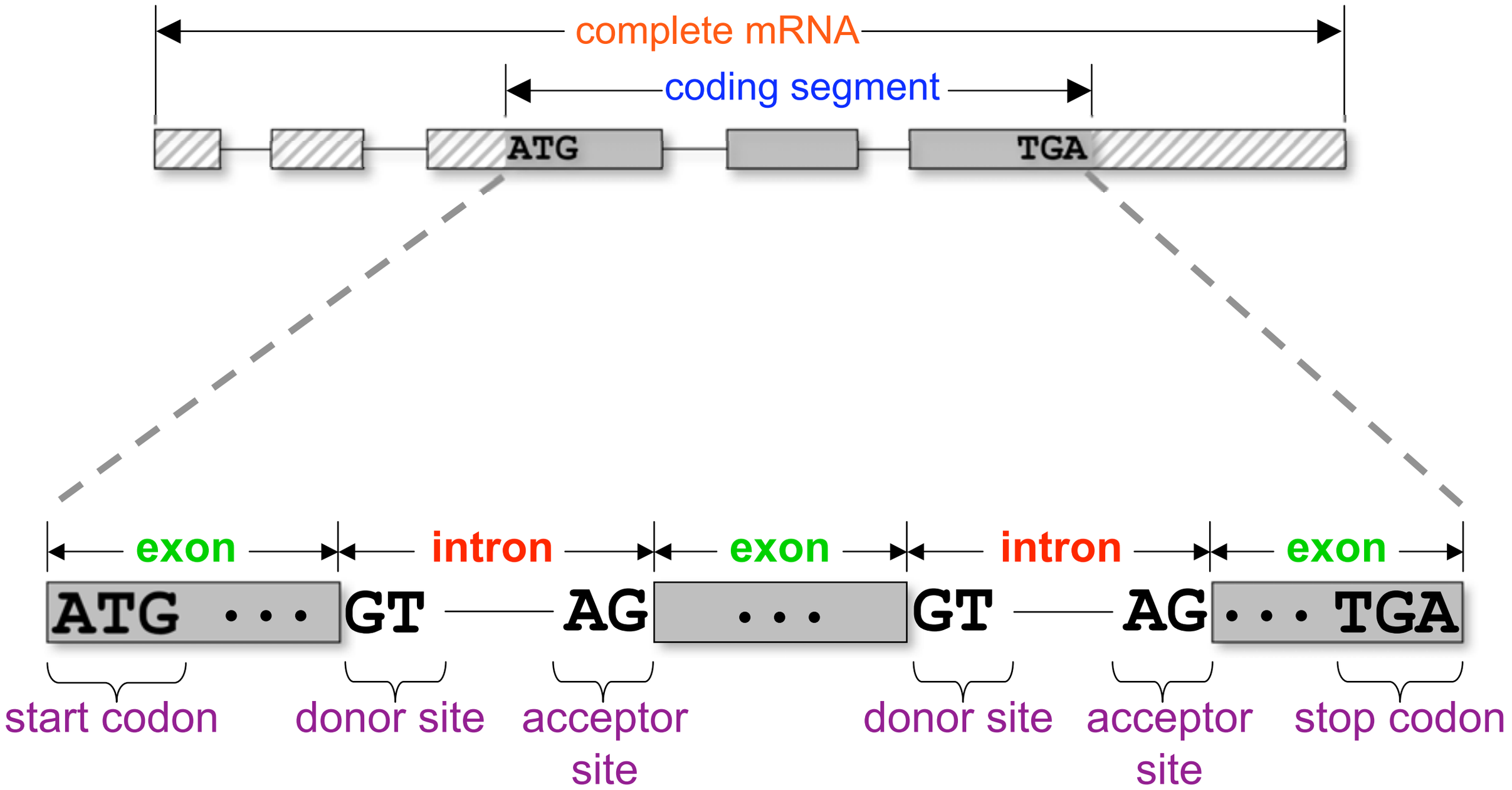


reverse strand



forward strand, spliced

The Eukaryotic Gene Finding Problem



The Stochastic Nature of Signal Sensing

(start codons)

A T G

(stop codons)

T G A
T A A
T A G

(donor splice sites)

G T

(acceptor splice sites)

A G

Common Assumptions in Gene Finding

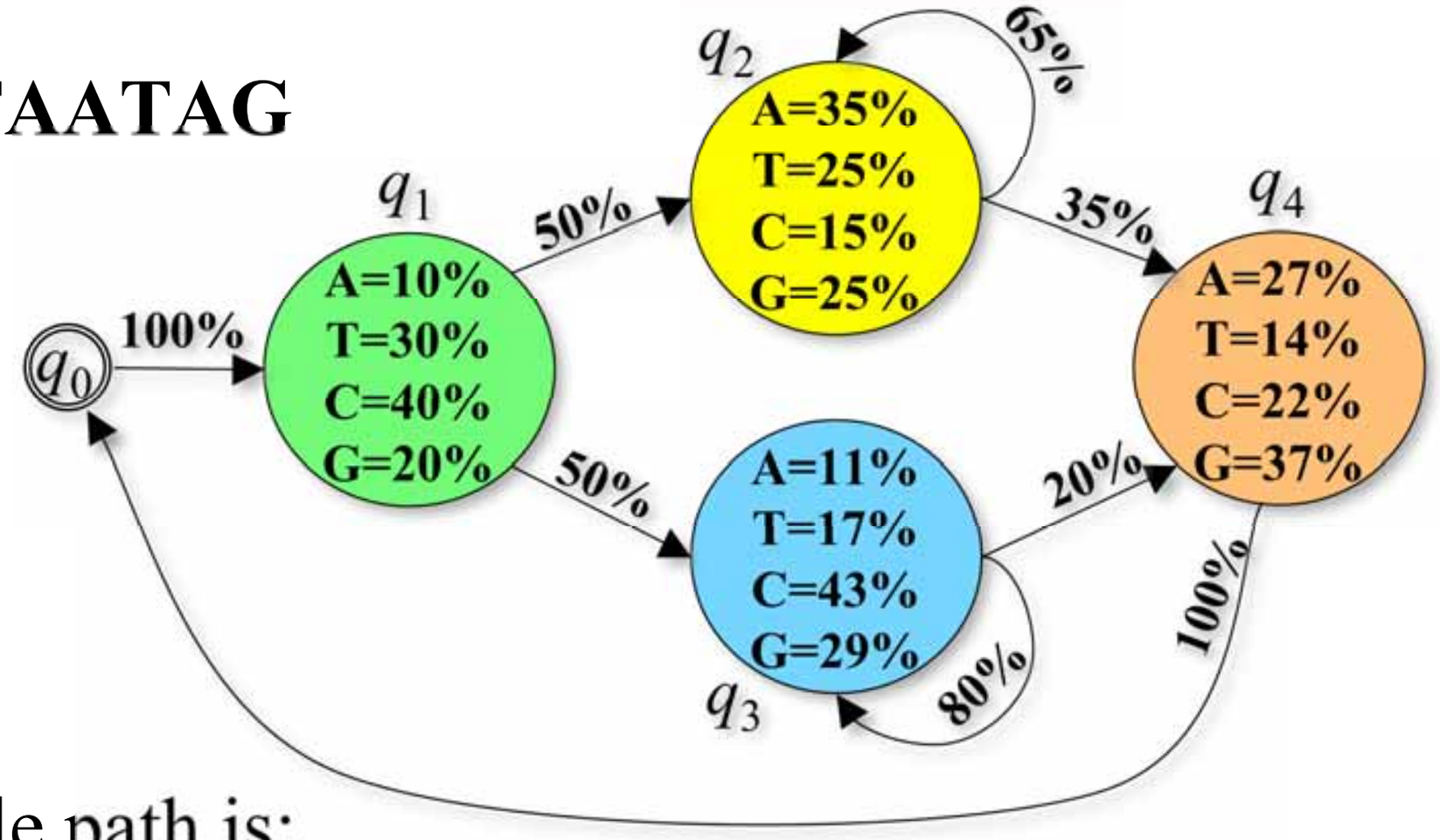
- No overlapping genes
- No nested genes
- No frame shifts or sequencing errors
- Optimal parse only
- No split start codons (ATGT...AGG)
- No split stop codons (TGT...AGAG)
- No alternative splicing
- No selenocysteine codons (TGA)
- No ambiguity codes (Y,R,N, etc.)

Finding the Most Probable Path

Example: **CATTAATAG**

top: 7.0×10^{-7}

bottom: 2.8×10^{-9}



The most probable path is:

States: 1 2 2 2 2 2 2 2 4

Sequence: C A T T A A T A G

resulting in this parse:

States: 1 2 2 2 2 2 2 2 4

Sequence: C ATTAATA G

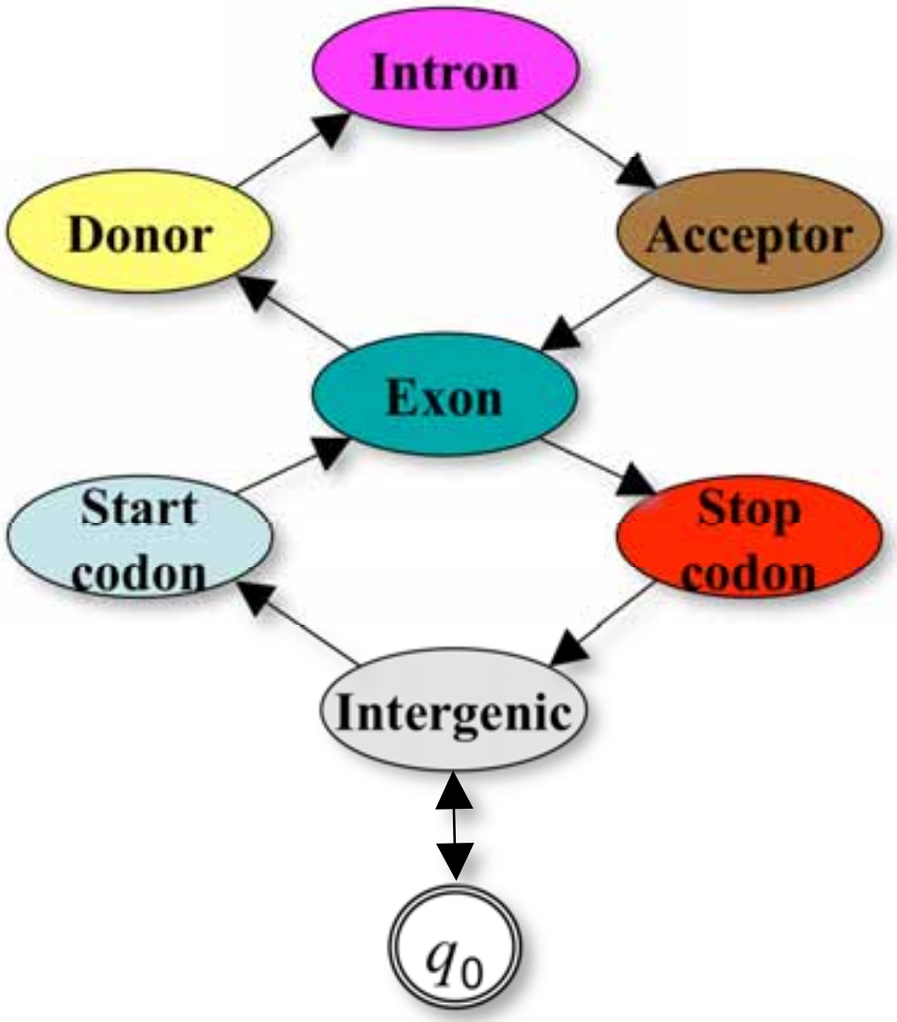
feature 1: C

feature 2: ATTAATA

feature 3: G

Using an HMM for Gene Prediction

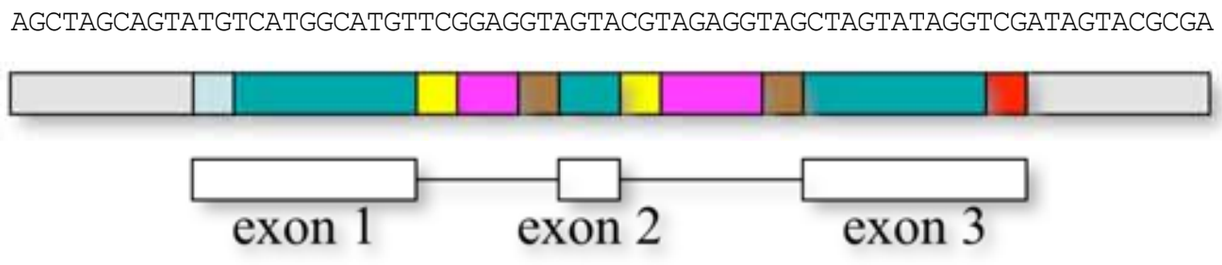
the Markov model:



the input sequence: AGCTAGCAGTATGTCATGGCATGTTCGGAGGTAGTACGTAGAGGTAGCTAGTATAGGTCGATAGTACGCGA

the most probable path:

the gene prediction:

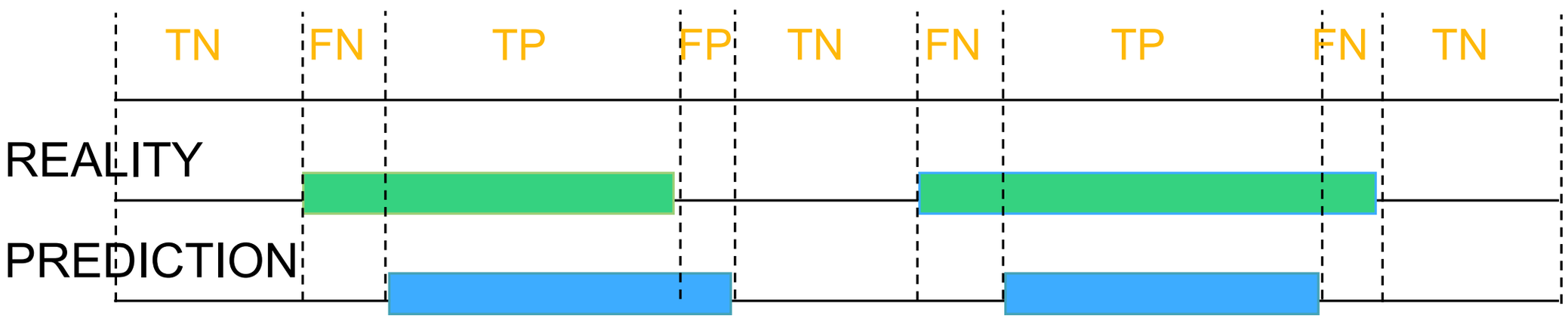


Higher Order Markovian Eukaryotic Recognizer (HOMER)

#

Evaluation of Gene Finding Programs

Nucleotide level accuracy



Sensitivity: $Sn = \frac{TP}{TP + FN}$ What fraction of reality did you predict?

Specificity: $Sp = \frac{TP}{TP + FP}$ What fraction of your predictions are real?

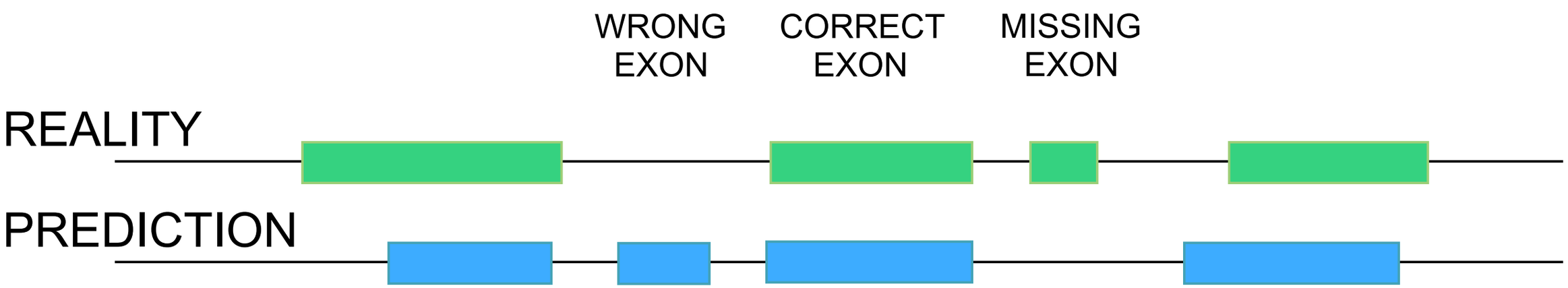
(actually, this is Precision, which is not the same thing)

Nonetheless, this measure is used below and is called “Specificity” in the gene-finding community. For more details, see Burset, Moises, and Roderic Guigo. "Evaluation of gene structure prediction programs." Genomics 34.3 (1996): 353-367.

* slide adapted from Mike Schatz

More Measures of Prediction Accuracy

Exon level accuracy



$$ExonSn = \frac{TE}{AE} = \frac{\text{number of correct exons}}{\text{number of actual exons}}$$

$$ExonSp = \frac{TE}{PE} = \frac{\text{number of correct exons}}{\text{number of predicted exons}}$$

Defined equiv. for splice sites & start/stop codons

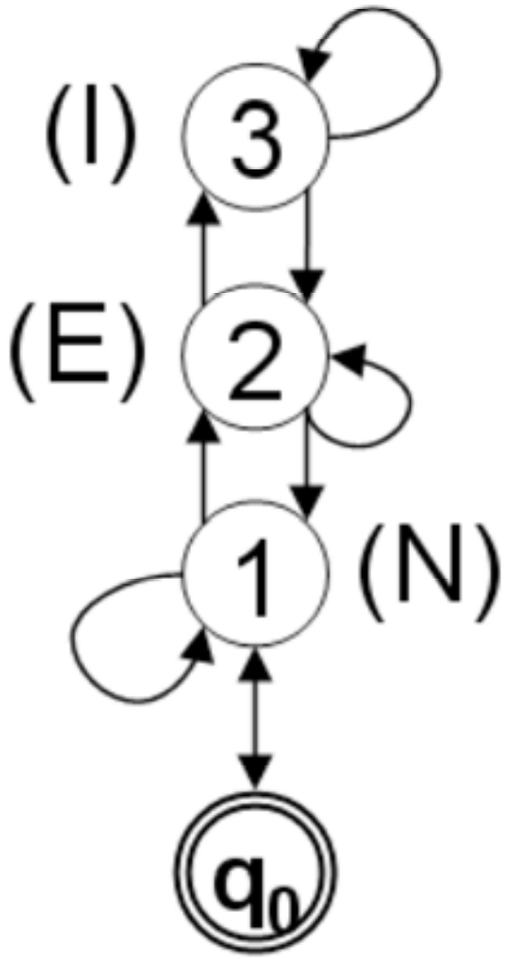
* slide from Mike Schatz

HOMER, version H_3

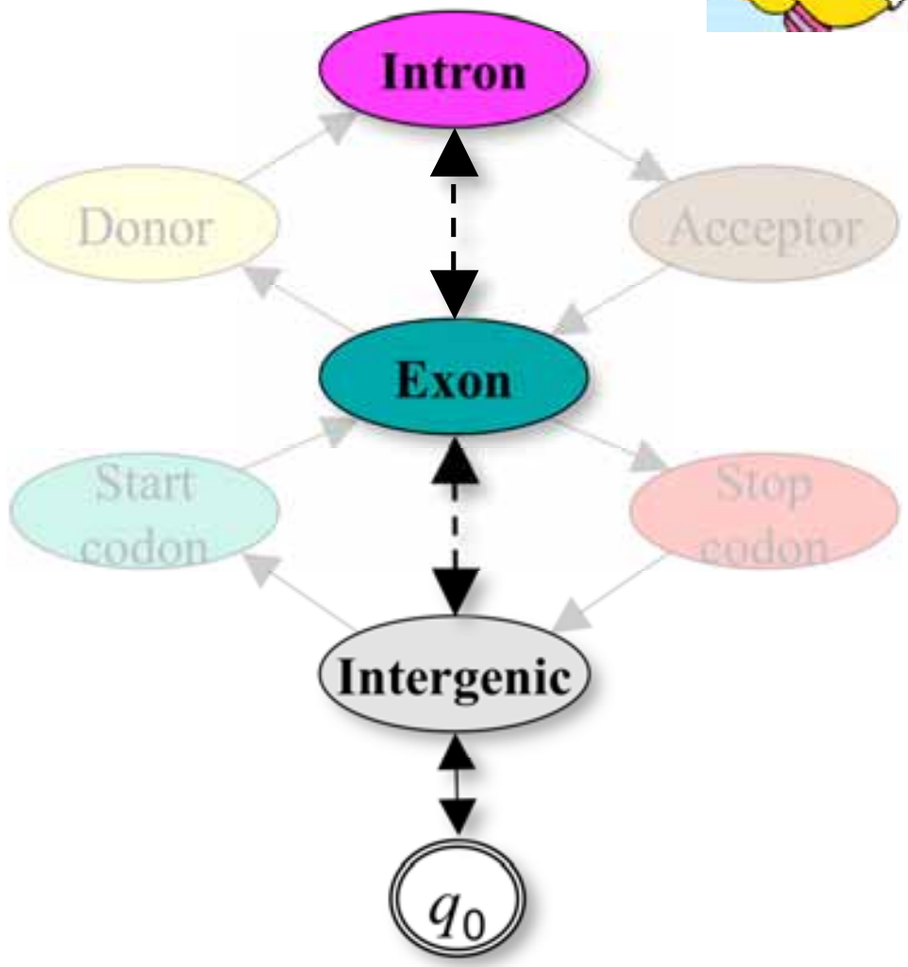
I=intron state

E=exon state

N=intergenic state



≈

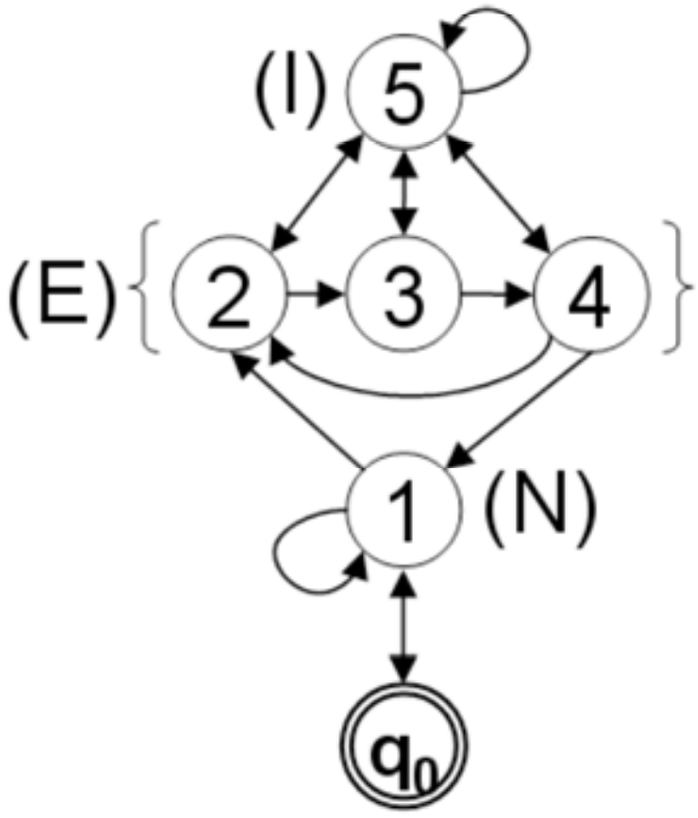


tested on 500
Arabidopsis genes:

	nucleotides			**splice sites**		**start/stop codons**		**exons**			**genes**	
	Sn	*Sp*	*F*	*Sn*	*Sp*	*Sn*	*Sp*	*Sn*	*Sp*	*F*	*Sn*	#
baseline	50	28	44	0	0	0	0	0	0	0	0	0
H_3	53	88	66	0	0	0	0	0	0	0	0	0

#

HOMER, version H_5



three exon states, for the three codon positions

	nucleotides			splice sites		start/stop codons		exons			genes	
	Sn	*Sp*	*F*	*Sn*	*Sp*	*Sn*	*Sp*	*Sn*	*Sp*	*F*	*Sn*	#
H_3	53	88	66	0	0	0	0	0	0	0	0	0
H_5	65	91	76	1	3	3	3	0	0	0	0	0

#

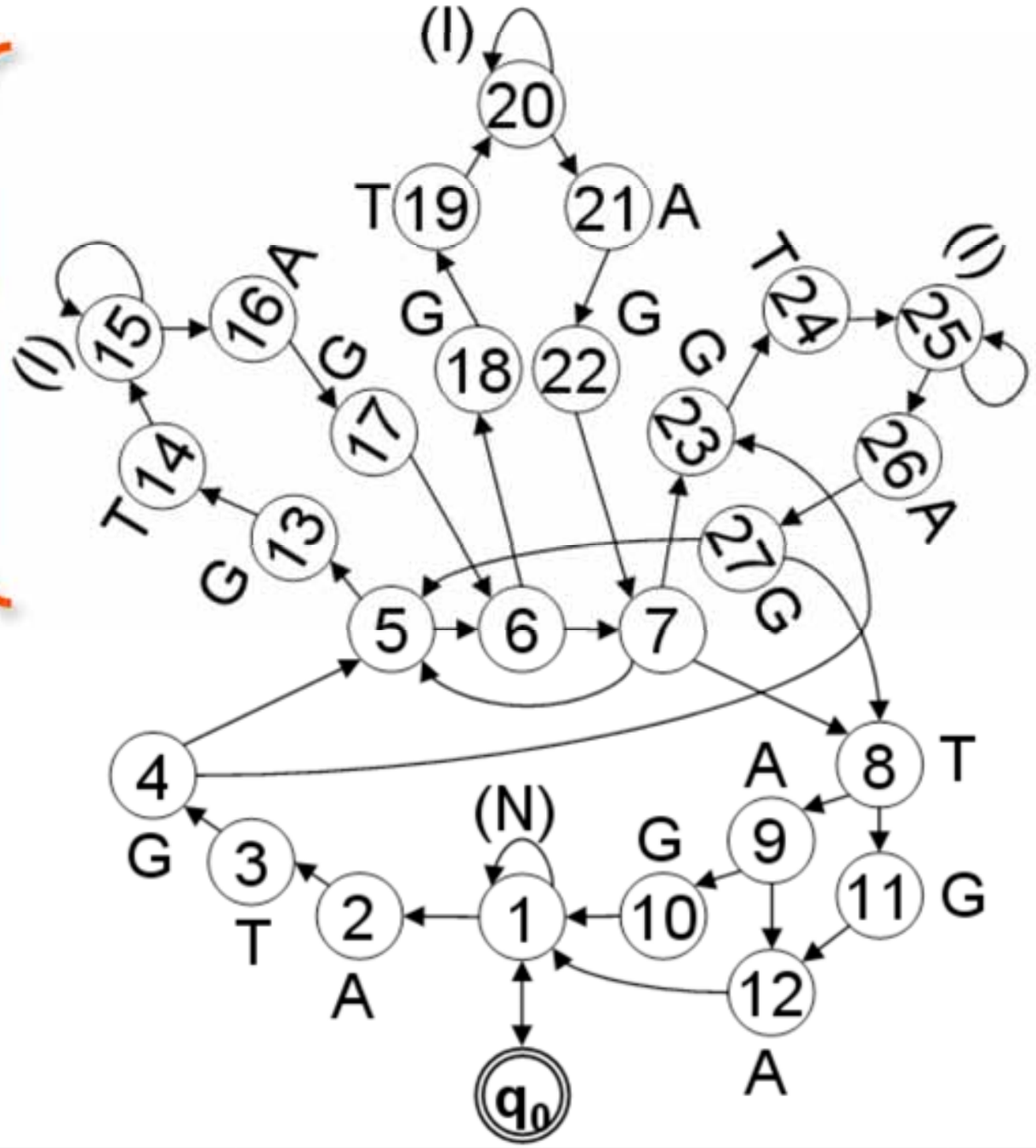
HOMER
version H_{17}

	nucleotides			splice sites		start/stop codons		exons			genes	
	Sn	*Sp*	*F*	*Sn*	*Sp*	*Sn*	*Sp*	*Sn*	*Sp*	*F*	*Sn*	#
H_5	65	91	76	1	3	3	3	0	0	0	0	0
H_{17}	81	93	87	34	48	43	37	19	24	21	7	35

#

HOMER version H_{27}

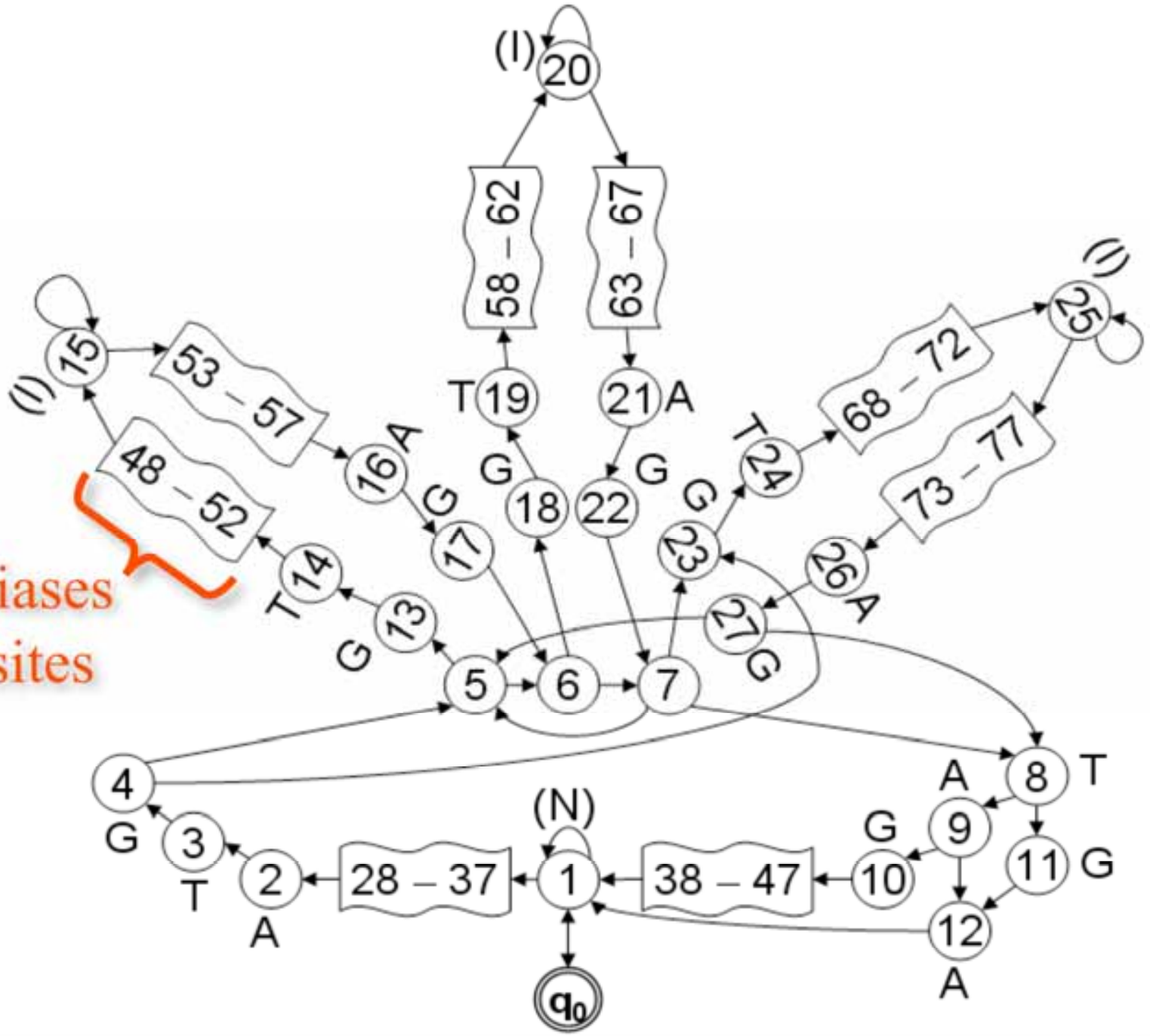
three separate intron models



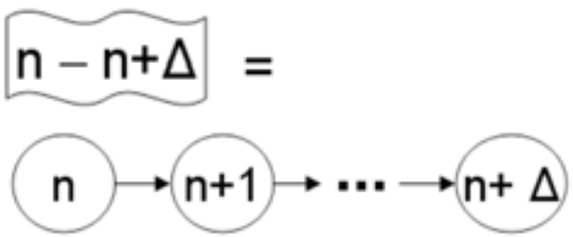
	nucleotides			splice		start/stop		exons			genes	
	Sn	*Sp*	*F*	*Sn*	*Sp*	*Sn*	*Sp*	*Sn*	*Sp*	*F*	*Sn*	#
H_{17}	81	93	87	34	48	43	37	19	24	21	7	35
H_{27}	83	93	88	40	49	41	36	23	27	25	8	38

#

HOMER
version H_{77}

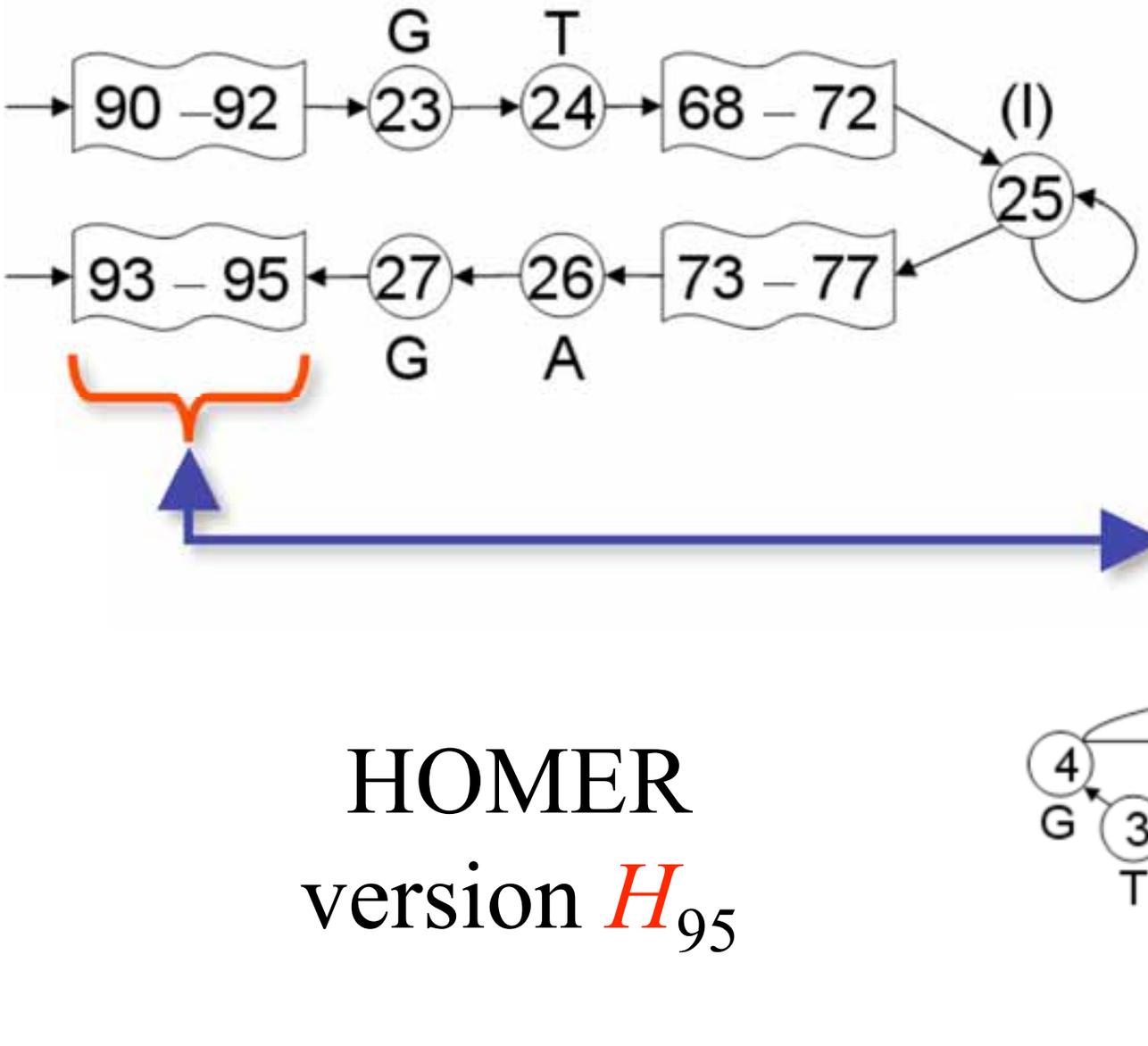


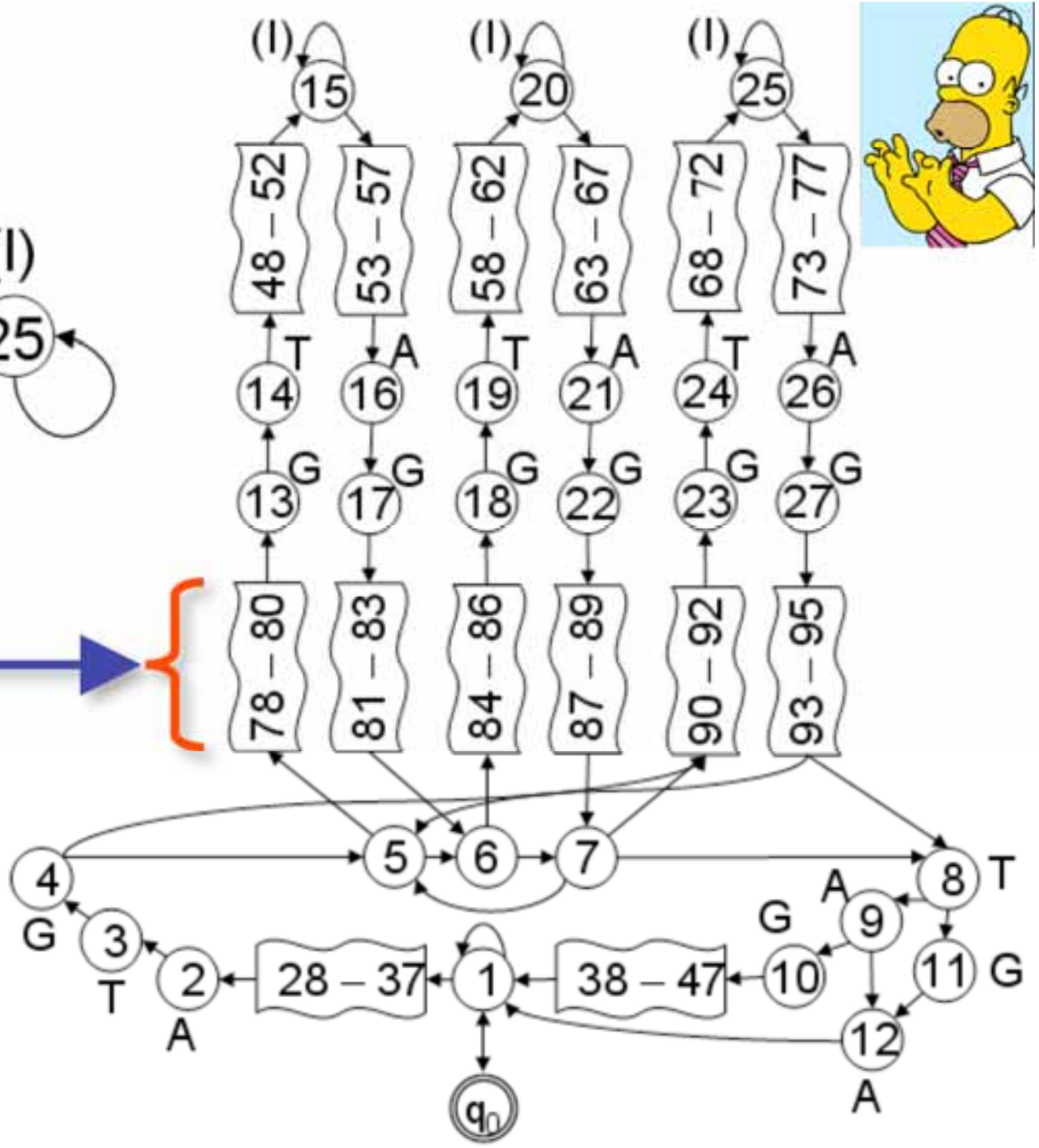
positional biases
near splice sites



	nucleotides			splice		start/stop		exons			genes	
	Sn	*Sp*	*F*	*Sn*	*Sp*	*Sn*	*Sp*	*Sn*	*Sp*	*F*	*Sn*	#
H_{27}	83	93	88	40	49	41	36	23	27	25	8	38
H_{77}	88	96	92	66	67	51	46	47	46	46	13	65

#





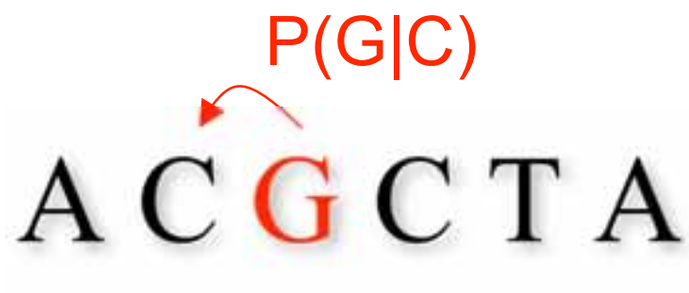
HOMER
version H_{95}

	nucleotides			splice		start/stop		exons			genes	
	Sn	*Sp*	*F*	*Sn*	*Sp*	*Sn*	*Sp*	*Sn*	*Sp*	*F*	*Sn*	#
H_{77}	88	96	92	66	67	51	46	47	46	46	13	65
H_{95}	92	97	94	79	76	57	53	62	59	60	19	93

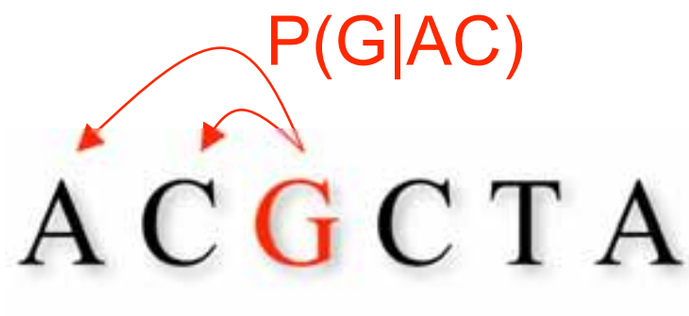
Higher-order Markov Models

0th order:

1st order:



2nd order:



Higher-order Markov Models

	order	nucleotides			splice sites		starts/ stops		exons			genes	
		Sn	*Sp*	*F*	*Sn*	*Sp*	*Sn*	*Sp*	*Sn*	*Sp*	*F*	*Sn*	#
H^0_{95}	0	92	97	94	79	76	57	53	62	59	60	19	93
H^1_{95}	1	95	98	97	87	81	64	61	72	68	70	25	127
H^2_{95}	2	98	98	98	91	82	65	62	76	69	72	27	136
H^3_{95}	3	98	98	98	91	82	67	63	76	69	72	28	140
H^4_{95}	4	98	97	98	90	81	69	64	76	68	72	29	143
H^5_{95}	5	98	97	98	90	81	66	62	74	67	70	27	137

#

Generalized HMMs

- Each state can emit a *sequence* of symbols.
- In the diagrams on the next few slides, each state emits a *complete gene feature* (e.g. an entire exon):

$$\max_{\pi} \prod_{i=1}^{n} \Pr(x_i \ldots x_{i+d_i} \mid \pi_i, d_i) \Pr(d_i \mid \pi_i) \Pr(\pi_i \to \pi_{i+1})$$

Probability that the state will emit d_i symbols.

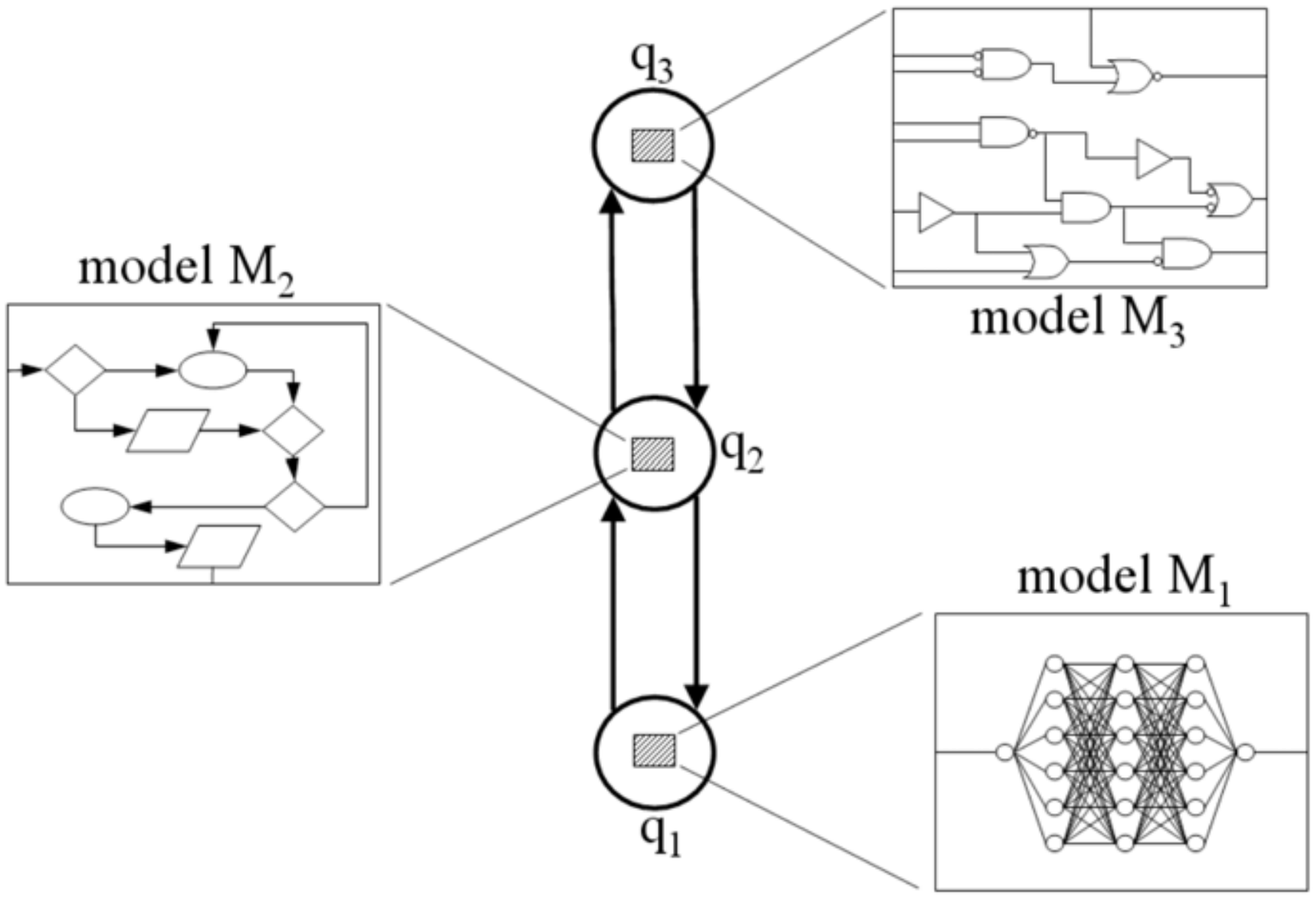
Probability of emitting the string of length d_i.

Probability of transitioning to the next state

This probability could itself be computed by an HMM or a Markov chain, etc.

* slide from Carl Kingsford

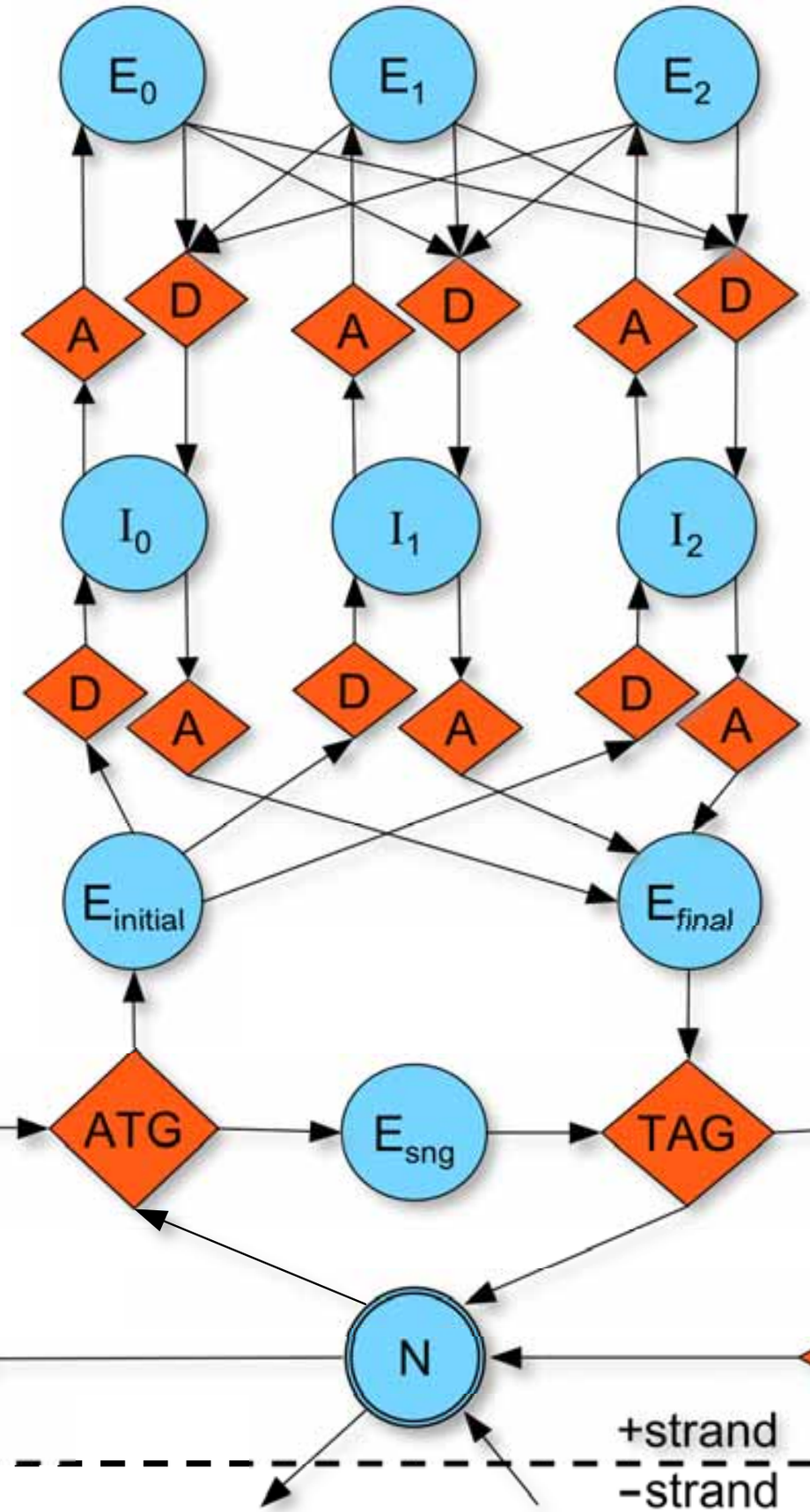
Generalized Hidden Markov Models



Advantages:	Disadvantages:
* Submodel abstraction * Architectural simplicity * State duration modeling	* Decoding complexity

Fixed-length states are called *signal states* (red).

Each state has a separate submodel or "sensor"



Variable-length states are called *content states* (blue).

Components Needed

- Probability distribution of initial state

  = the fraction of known genome corresponding to each state, divided into groups by GC content.

- State transition probabilities

  = the probability X follows Y in known genes

- Length distributions for each state

  For exons: = estimated from empirically observed distribution

  For introns: = geometric distribution with parameter q_{gc}, where is the best fit parameter for regions with a given GC content.

- Sequence models for each state/length

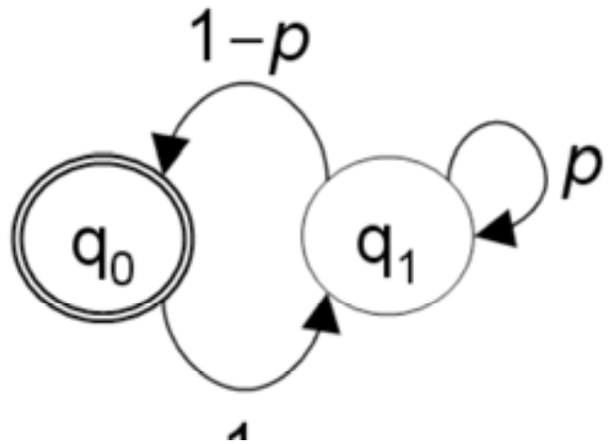
  for states with strong motifs:

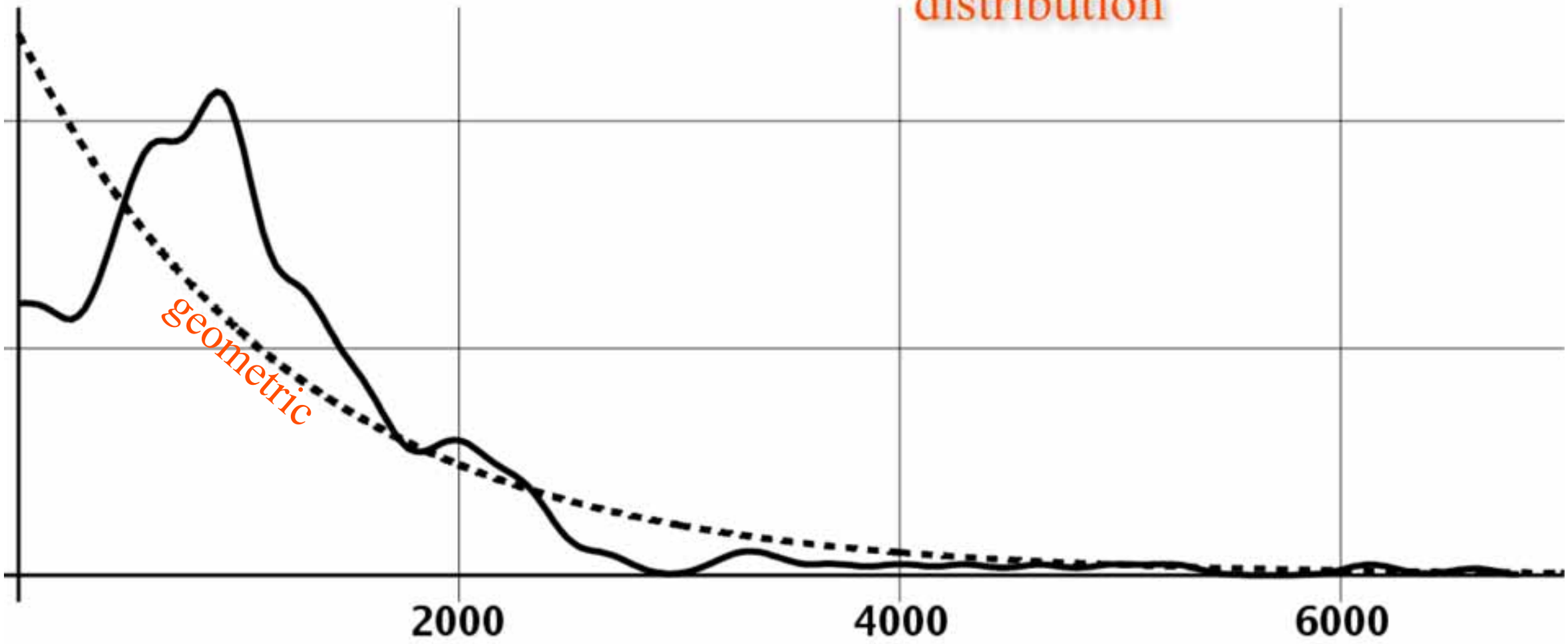
* slide from Carl Kingsford

HMMs & Geometric Feature Lengths

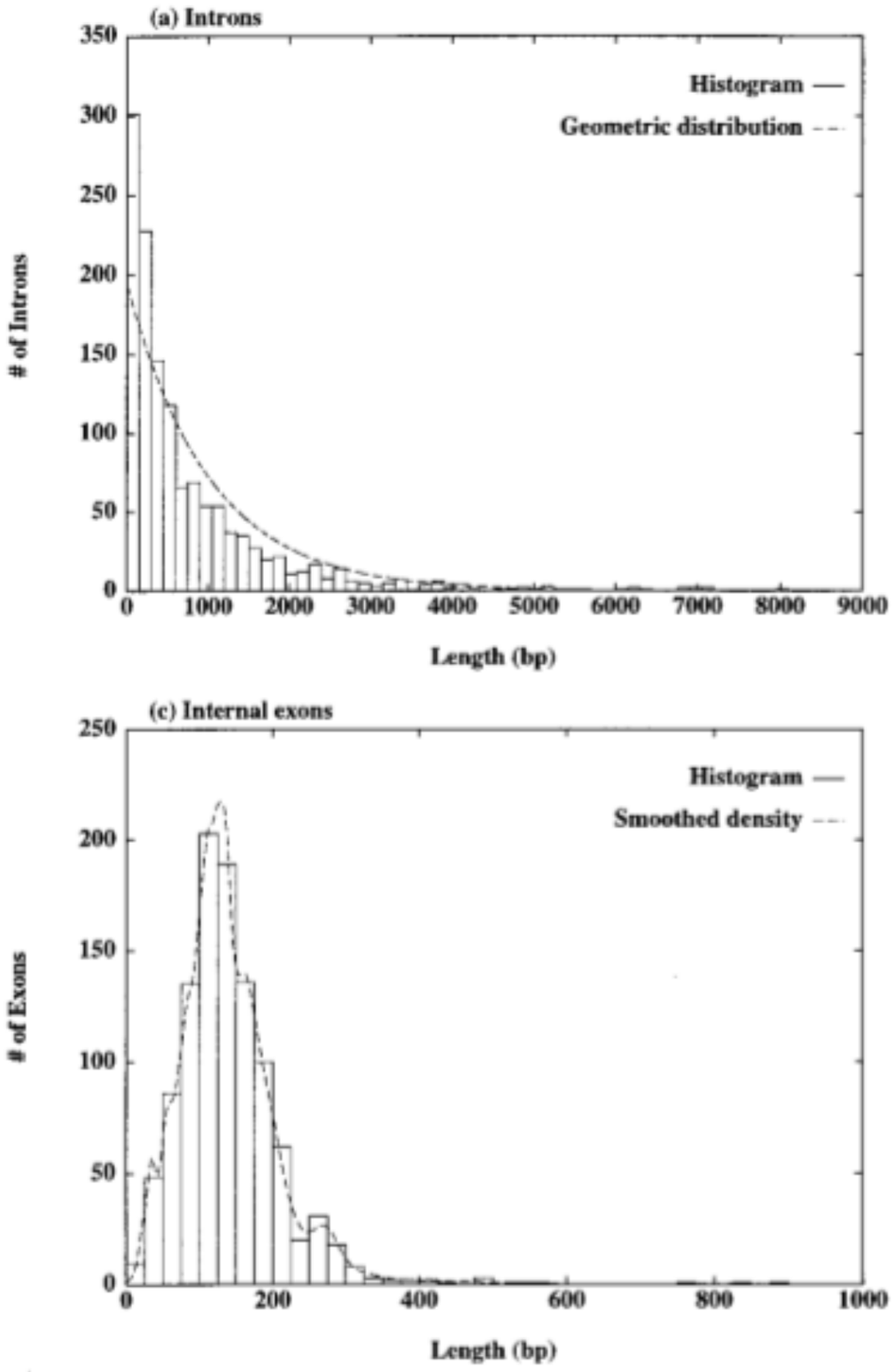
$$P(x_0 \ldots x_{d-1} \mid \theta) = \left(\prod_{i=0}^{d-1} P_e(x_i \mid \theta) \right) p^{d-1}(1-p)$$

geometric distribution





Feature Length Distributions



Decoding with a GHMM

$$\phi_{\max} = \underset{\phi}{argmax}\, P(\phi \mid S) = \underset{\phi}{argmax}\, \frac{P(\phi \wedge S)}{P(S)}$$

$$= \underset{\phi}{argmax}\, P(\phi \wedge S)$$

$$= \underset{\phi}{argmax}\, P(S \mid \phi) P(\phi)$$

$$P(S \mid \phi) = \prod_{i=1}^{|\phi|-2} P_e(S_i \mid q_{(i)}, d_i)$$

emission prob.

$$P(\phi) = \prod_{i=0}^{|\phi|-2} P_t(q_{(i+1)} \mid q_{(i)}) P_d(d_i \mid q_{(i)})$$

transition prob.

duration prob.

$$\phi_{\max} = \underset{\phi}{argmax} \prod_{i=0}^{|\phi|-2} P_e(S_i \mid q_{(i)}, d_i) P_t(q_{(i+1)} \mid q_{(i)}) P_d(d_i \mid q_{(i)})$$

Accuracy

	nucleotides			splice		start/stop		exons			genes	
	Sn	*Sp*	*F*	*Sn*	*Sp*	*Sn*	*Sp*	*Sn*	*Sp*	*F*	*Sn*	#
HMM	98	97	98	90	81	66	62	74	67	70	27	
GHMM	94	96	95	87	89	77	74	79	80	80	45	

#

GlimmerHMM (a *generalized* HMM for gene finding)

% of predicted in-gene nucleotides that are correct

% of predicted exons that are true exons.

	Nuc Sens	*Nuc Prec*	*Nuc Accur*	*Exon Sens*	*Exon Prec*	*Exact Genes*	*Size of test set*
D.rerio	93%	78%	86%	77%	69%	24%	549 genes
C.elegans	96%	95%	96%	82%	81%	42%	1886 genes
Arabidopsis	97%	99%	98%	84%	89%	60%	809 genes
Cryptococcus	96%	99%	98%	86%	88%	53%	350 genes
Coccidioides	99%	99%	99%	84%	86%	60%	503 genes
Brugia	93%	98%	95%	78%	83%	25%	477 genes

% of true gene nucleotides that GlimmerHMM predicts as part of genes.

% of true exons that GlimmerHMM found.

% of genes perfectly found

* slide from Carl Kingsford

Compare with GENSCAN (another generalized HMM)

- On 963 human genes:

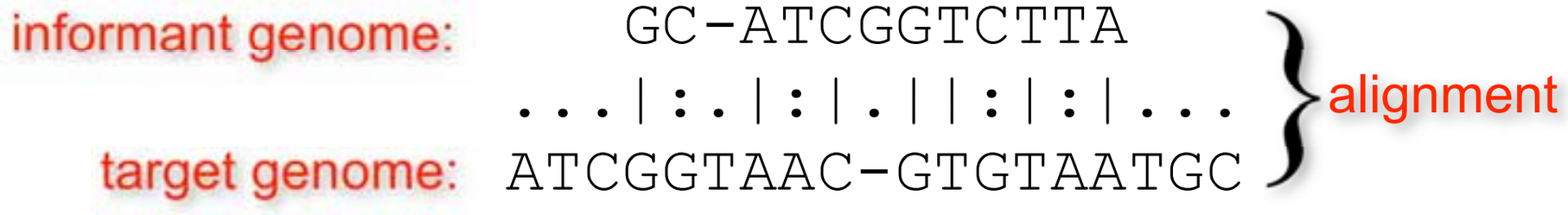
	Nuc Sens	*Nuc Prec*	*Nuc Acc*	*Exon Sens*	*Exon Prec*	*Exon Acc*	*Exact Genes*
GlimmerHMM	86%	72%	79%	72%	62%	67%	17%
Genscan	86%	68%	77%	69%	60%	65%	13%

- **Note** that overall accuracy is pretty low.

* slide from Carl Kingsford

Comparative Methods

Problem: Predict genes in a *target genome* G based on the contents of G and also based on the contents of one or more *informant genomes* $I^{(1)}$... $I^{(n)}$:



Rationale: *Natural selection should operate more strongly on protein-coding DNA than on non-functional DNA such as introns.*

Pair Generalized HMMs

Use: find genes simultaneously in 2 genomes
increased signal b/c the structure of homologous genes is often very similar.

- Pair: Each state emits two symbols, one for each sequence
- Generalized Pair: a pair of lengths d, e is drawn from a joint probability distribution and a pair of sequences X,Y of length d,e, respectively, are generated at each state.

Pachter et al. J Comp Biol, 9(2), 2002

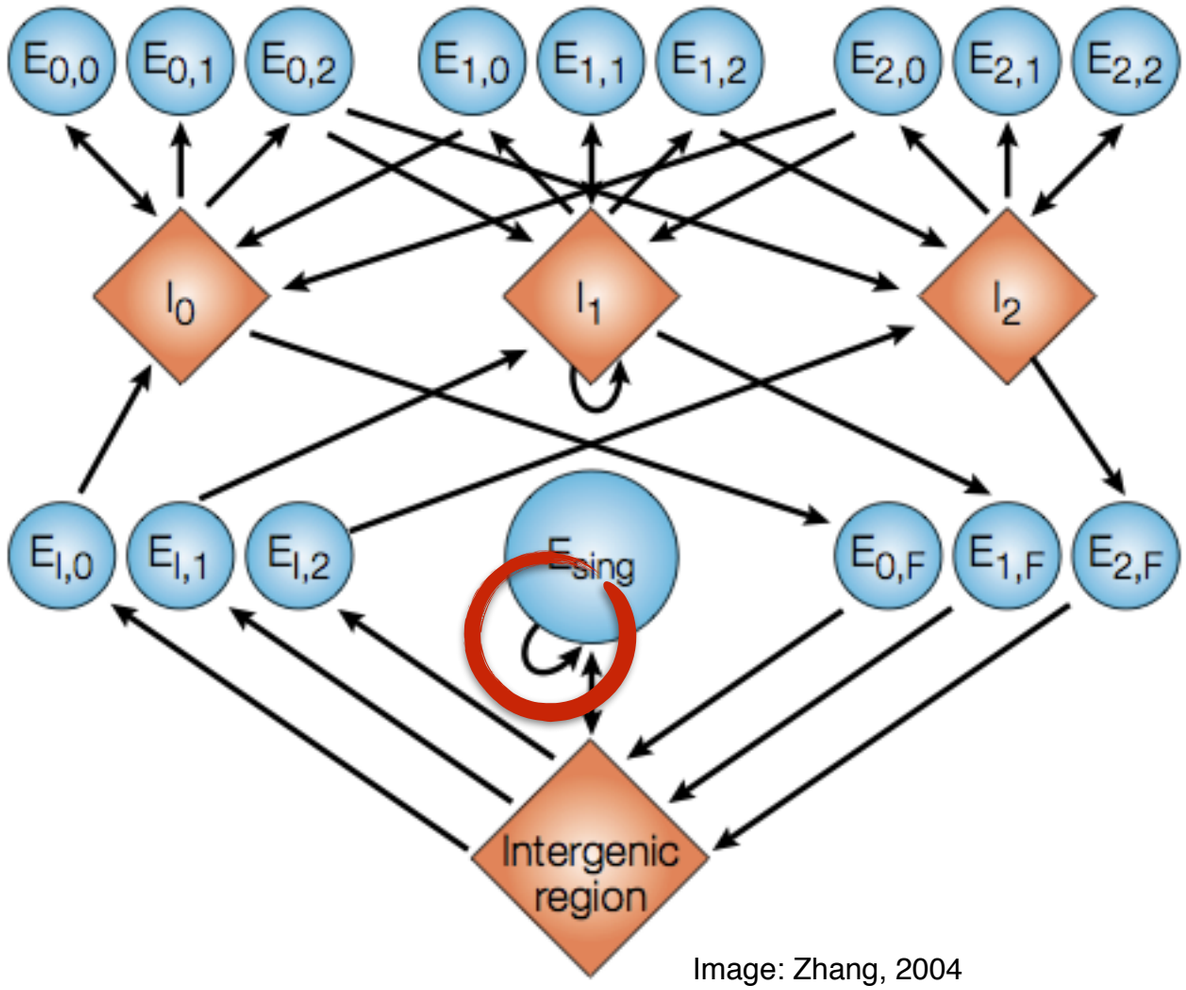


Image: Zhang, 2004

Reverse strand: mirror reflection of above

* slide from Carl Kingsford

Accuracy

Data set: 147 high-confidence *Aspergillus fumigatus* × *A. nidulans* orthologs (493 exons, 564kb).

	nucleotide accuracy	exon sensitivity	exon specificity	exact genes
GHMM	99%	78%	73%	54%
GPHMM	99%	89%	85%	74%

How Does Homology Help?

feature	amino acid alignment score	<,>	nucleotide alignment score
exon 1	100%	>	71%
intron 1	14%	<	51%
exon 2	98%	>	85%
intron 2	29%	<	49%
exon 3	97%	>	82%
intron 3	9%	<	49%
exon 4	96%	>	83%

#

Recap

- Simple gene finding approaches use codon bias and long ORFs to identify genes.

- Many top gene finding programs for Eukaryotes are based on generalizations of Hidden Markov Models because multiple types of signals are present in a gene (intron, exon, etc.)

- Basic HMMs must be generalized in many way (e.g. to emit variable sized strings, to emit two strings simultaneously, etc.).

* slide from Carl Kingsford