Interpolated Markov Models for Gene Finding

BMI/CS 776

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Goals for Lecture

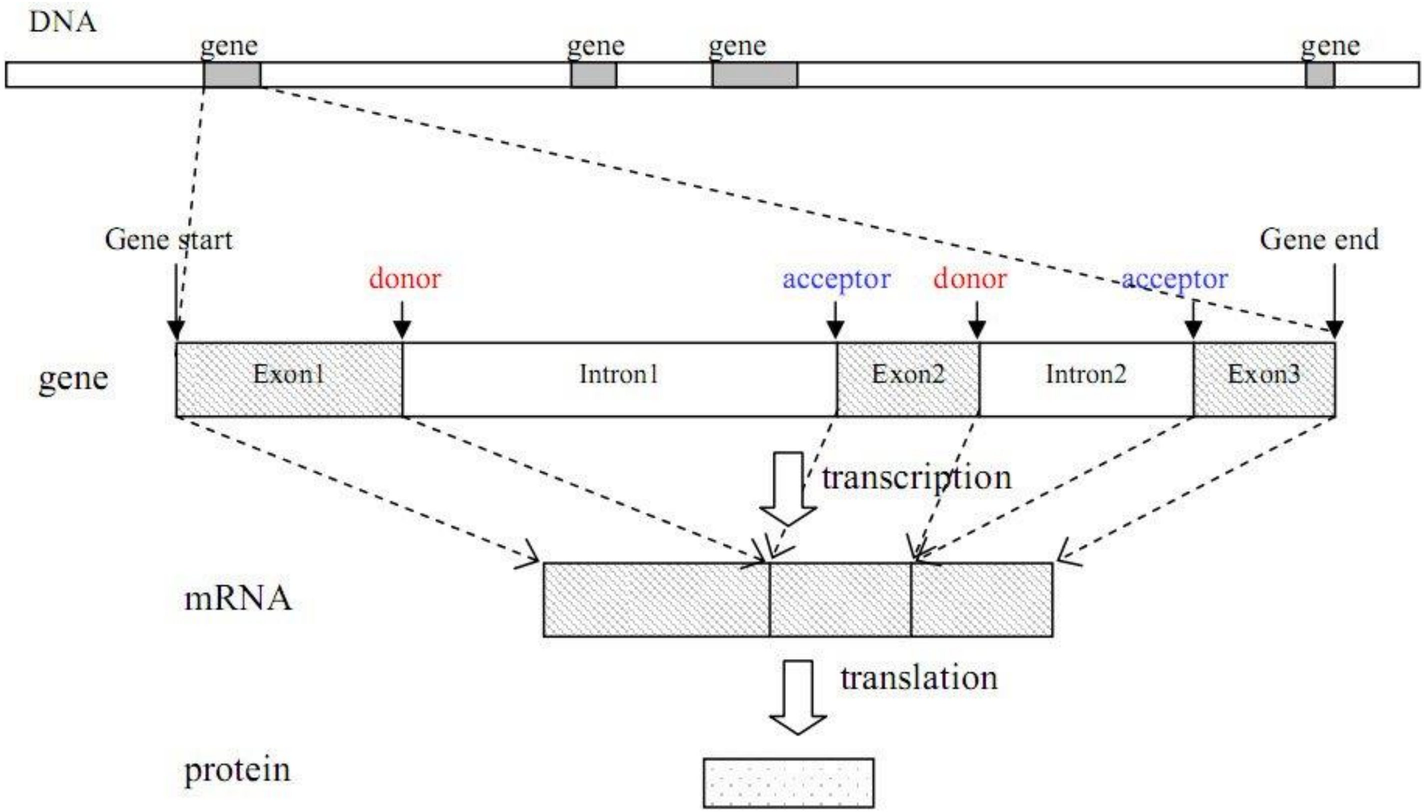
Key concepts

- the gene-finding task
- the trade-off between potential predictive value and parameter uncertainty in choosing the order of a Markov model
- interpolated Markov models

The Gene Finding Task

Given: an uncharacterized DNA sequence

Do: locate the genes in the sequence, including the coordinates of individual *exons* and *introns*



Sources of Evidence for Gene Finding

- **Signals**: the sequence *signals* (e.g. splice junctions) involved in gene expression

- **Content**: statistical properties that distinguish protein-coding DNA from non-coding DNA

- **Conservation**: signal and content properties that are conserved across related sequences (e.g. orthologous regions of the mouse and human genome)

Gene Finding: Search by Content

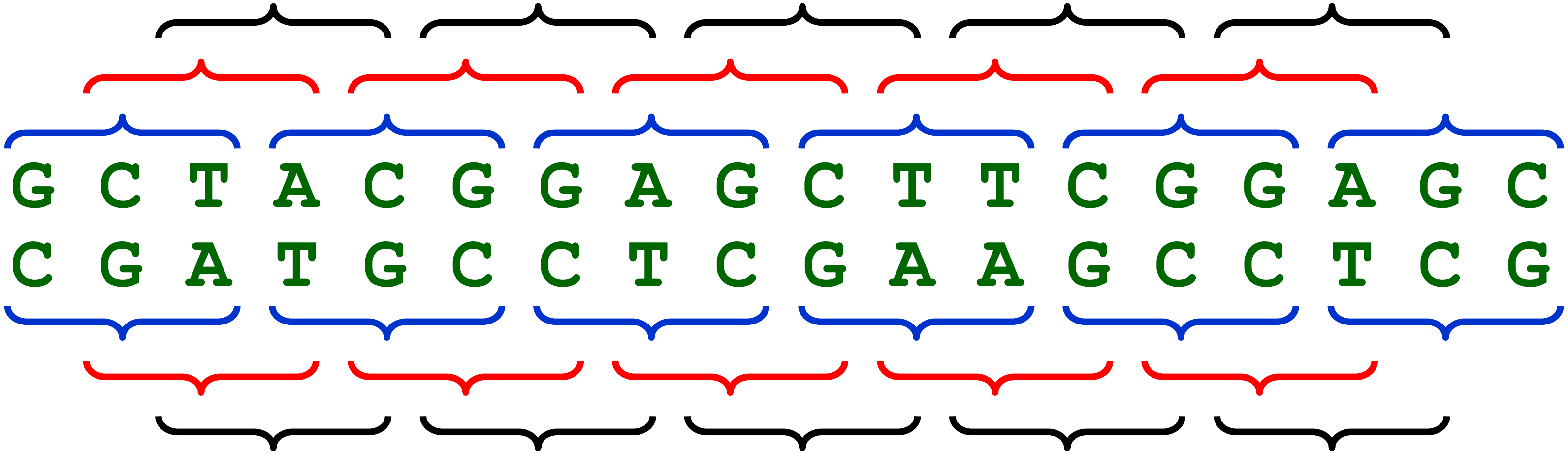
- Encoding a protein affects the statistical properties of a DNA sequence
  - some amino acids are used more frequently than others (Leu more popular than Trp)
  - different numbers of codons for different amino acids (Leu has 6, Trp has 1)
  - for a given amino acid, usually one codon is used more frequently than others
    - this is termed *codon preference*
    - these preferences vary by species

Codon Preference in E. Coli

AA	codon	/1000
Gly	GGG	1.89
Gly	GGA	0.44
Gly	GGU	52.99
Gly	GGC	34.55
Glu	GAG	15.68
Glu	GAA	57.20
Asp	GAU	21.63
Asp	GAC	43.26

Reading Frames

- A given sequence may encode a protein in any of the six reading frames

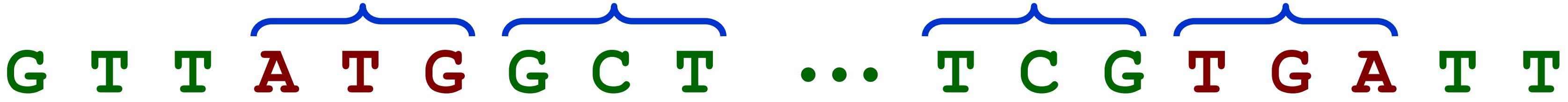


G C T A C G G A G C T T C G G A G C

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

Open Reading Frames (ORFs)

- An ORF is a sequence that
  - starts with a potential start codon
  - ends with a potential stop codon, *in the same reading frame*
  - doesn’t contain another stop codon in-frame
  - and is sufficiently long (say > 100 bases)

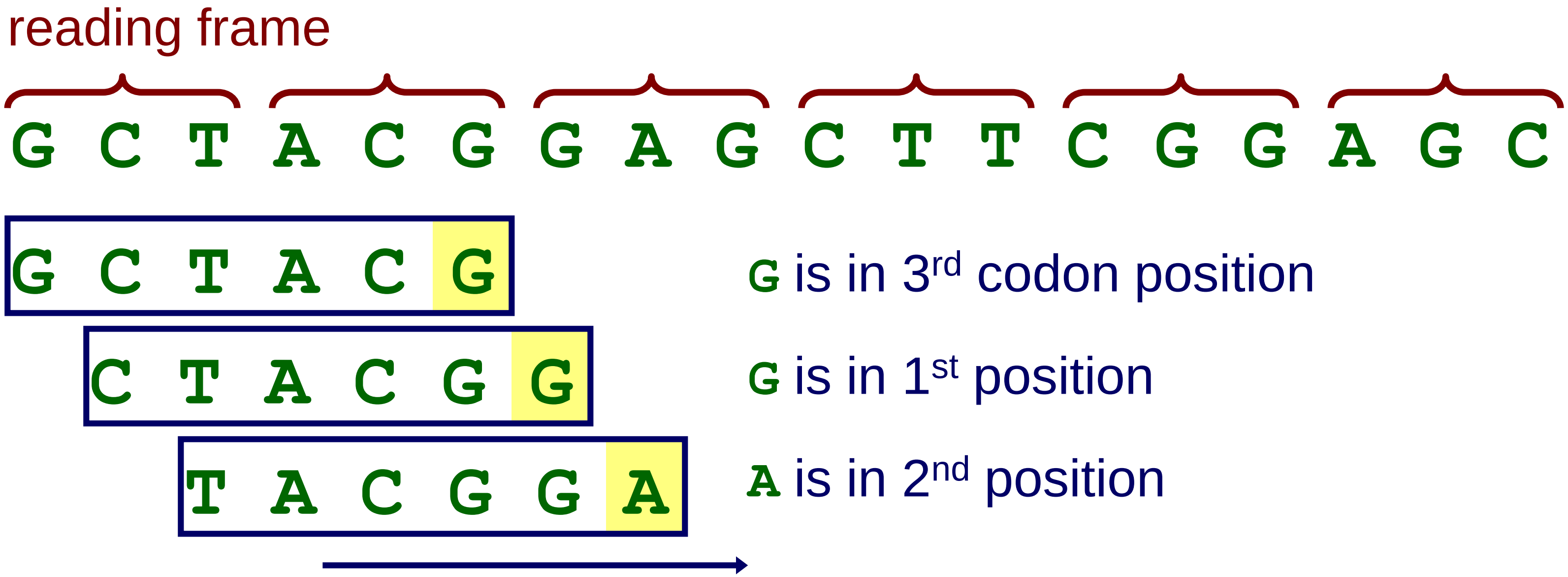


G T T A T G G C T ••• T C G T G A T T

- An ORF meets the minimal requirements to be a protein-coding gene in an organism without introns

Markov Models & Reading Frames

- Consider modeling a given coding sequence
- For each "word" we evaluate, we'll want to consider its position with respect to the reading frame we're assuming



reading frame

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

G C T A C G

C T A C G G

T A C G G A

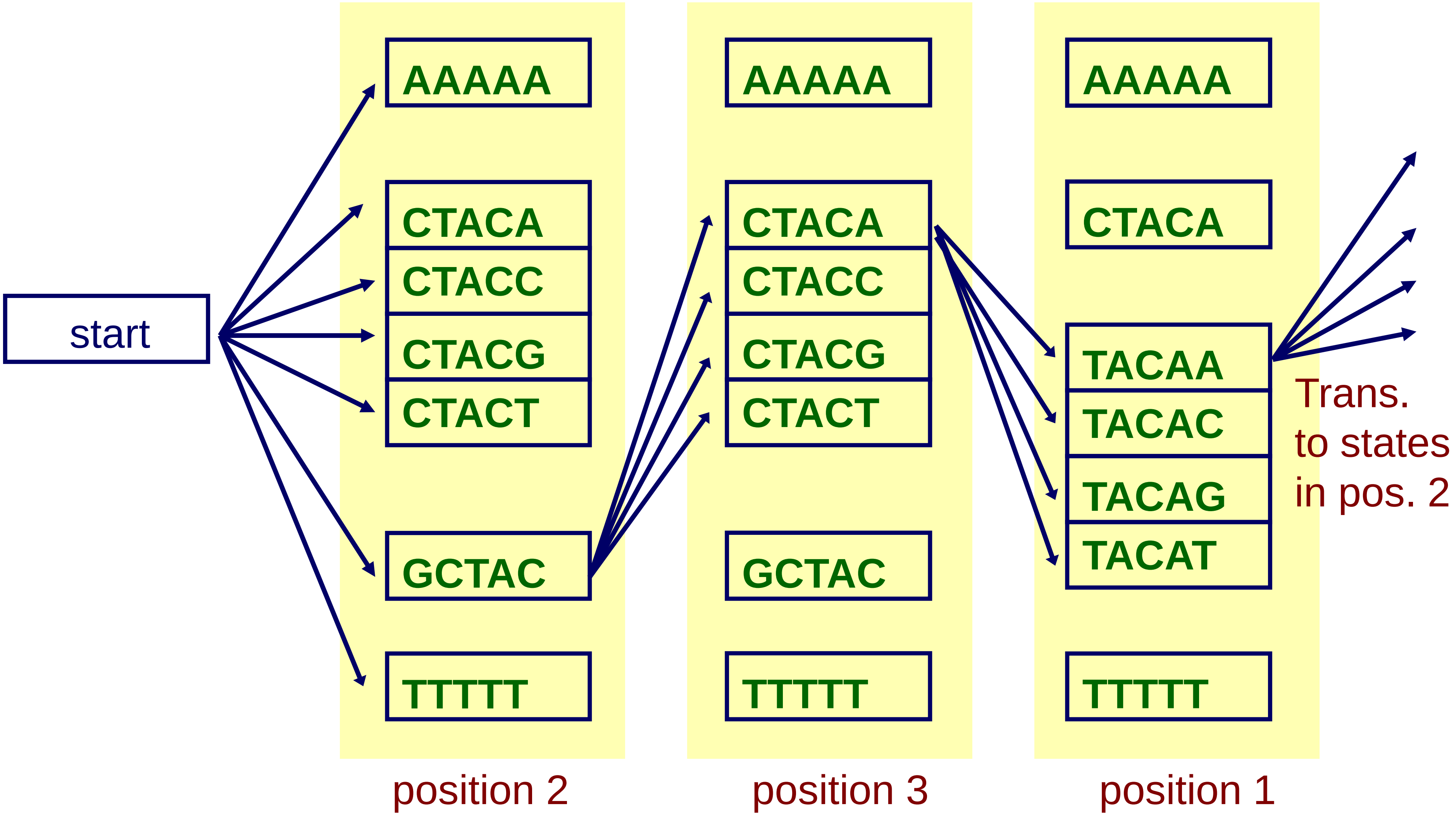
G is in 3rd codon position

G is in 1st position

A is in 2nd position

- Can do this using an inhomogeneous model

A Fifth Order Inhomogeneous Markov Chain



Selecting the Order of a Markov Chain Model

- Higher order models remember more “history”
- Additional history can have predictive value
- Example:
  - predict the next word in this sentence fragment “…you__” (are, give, passed, say, see, too, …?)
  - now predict it given more history

“…can you___”

“…say can you___”

“…oh say can you___”

Selecting the Order of a Markov Chain Model

- But the number of parameters we need to estimate grows exponentially with the order
  - for modeling DNA we need $O(4^{n+1})$ parameters for an *n*th order model

- The higher the order, the less reliable we can expect our parameter estimates to be
- Suppose we have 100k bases of sequence to estimate parameters of a model
  - for a 2nd order homogeneous Markov chain, we'd see each history 6250 times on average
  - for an 8th order chain, we'd see each history ~ 1.5 times on average

Interpolated Markov Models

- The IMM idea: manage this trade-off by interpolating among models of various orders
- *Simple* linear interpolation:

$$
\begin{aligned}
P_{\text{IMM}}(x_i \mid x_{i-n},\ldots,x_{i-1}) = {} & \lambda_0 P(x_i) \\
& + \lambda_1 P(x_i \mid x_{i-1}) \\
& \ldots \\
& + \lambda_n P(x_i \mid x_{i-n},\ldots,x_{i-1})
\end{aligned}
$$

- where $\sum_i \lambda_i = 1$

Interpolated Markov Models

- We can make the weights depend on the history
  - for a given order, we may have significantly more data to estimate some words than others
- *General* linear interpolation

$$
\begin{aligned}
P_{\text{IMM}}(x_i \mid x_{i-n},\ldots,x_{i-1}) = {} & \lambda_0 P(x_i) \\
& + \lambda_1(x_{i-1}) P(x_i \mid x_{i-1}) \\
& \ldots \\
& + \lambda_n(x_{i-n},\ldots,x_{i-1}) P(x_i \mid x_{i-n},\ldots,x_{i-1})
\end{aligned}
$$

λ is a function of the given history

The GLIMMER System

[Salzberg et al., Nucleic Acids Research, 1998]

- System for identifying genes in bacterial genomes
- Uses 8th order, inhomogeneous, interpolated Markov chain models

IMMs in GLIMMER

- How does GLIMMER determine the λ values?
- First, let’s express the IMM probability calculation recursively

$$P_{\mathrm{IMM,n}}(x_i \mid x_{i-n},\ldots,x_{i-1}) =$$
$$\lambda_{\mathrm{n}}(x_{i-n},\ldots,x_{i-1})P(x_i \mid x_{i-n},\ldots,x_{i-1}) +$$
$$[1-\lambda_n(x_{i-n},\ldots,x_{i-1})]P_{\mathrm{IMM,n\text{-}1}}(x_i \mid x_{i-n+1},\ldots,x_{i-1})$$

- Let $c(x_{i-n},\ldots,x_{i-1})$ be the number of times we see the history $x_{i-n},\ldots,x_{i-1}$ in our training set

$$\lambda_n(x_{i-n},\ldots,x_{i-1}) = 1 \quad \text{if} \quad c(x_{i-n},\ldots,x_{i-1}) > 400$$

IMMs in GLIMMER

- If we haven’t seen $x_{i-n},\ldots,x_{i-1}$ more than 400 times, then compare the counts for the following:

*n*th order history + base	(*n-1*)th order history + base
$x_{i-n},\ldots,x_{i-1},a$	$x_{i-n+1},\ldots,x_{i-1},a$
$x_{i-n},\ldots,x_{i-1},c$	$x_{i-n+1},\ldots,x_{i-1},c$
$x_{i-n},\ldots,x_{i-1},g$	$x_{i-n+1},\ldots,x_{i-1},g$
$x_{i-n},\ldots,x_{i-1},t$	$x_{i-n+1},\ldots,x_{i-1},t$

- Use a statistical test (χ^2) to get a value d indicating our confidence that the distributions of x_i depend on the order

IMMs in GLIMMER

- Putting it all together

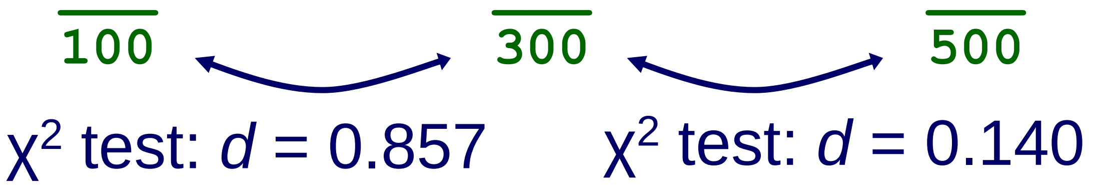
$$\lambda_n(x_{i-n},\ldots,x_{i-1}) = \begin{cases} 1 & \text{if } c(x_{i-n},\ldots,x_{i-1}) > 400 \\ d \times \dfrac{c(x_{i-n},\ldots,x_{i-1})}{400} & \text{else if } d \geq 0.5 \\ 0 & \text{otherwise} \end{cases}$$

where $d \in (0,1)$

IMM Example

- Suppose we have the following counts from our training set

ACGA	25	CGA	100	GA	175
ACGC	40	CGC	90	GC	140
ACGG	15	CGG	35	GG	65
ACGT	20	CGT	75	GT	120
	100		300		500



χ^2 test: $d = 0.857$

χ^2 test: $d = 0.140$

$\lambda_3(\textbf{ACG}) = 0.857 \times 100/400 = 0.214$

$\lambda_2(\textbf{CG}) = 0 \quad (d < 0.5,\ c(\textbf{CG}) < 400)$

$\lambda_1(\textbf{G}) = 1 \quad (c(\textbf{G}) > 400)$

IMM Example (Continued)

- Now suppose we want to calculate $P_{\mathrm{IMM},3}(T \mid ACG)$

$$\begin{aligned} P_{\mathrm{IMM},1}(T \mid G) &= \lambda_1(G)P(T \mid G) + \left(1-\lambda_1(G)\right)P_{\mathrm{IMM},0}(T) \\ &= P(T \mid G) \end{aligned}$$

$$\begin{aligned} P_{\mathrm{IMM},2}(T \mid CG) &= \lambda_2(CG)P(T \mid CG) + \left(1-\lambda_2(CG)\right)P_{\mathrm{IMM},1}(T \mid G) \\ &= P(T \mid G) \end{aligned}$$

$$\begin{aligned} P_{\mathrm{IMM},3}(T \mid ACG) &= \lambda_3(ACG)P(T \mid ACG) + \left(1-\lambda_3(ACG)\right)P_{\mathrm{IMM},2}(T \mid CG) \\ &= 0.214 \times P(T \mid ACG) + (1-0.214) \times P(T \mid G) \\ &= 0.214 \times 0.2 + (1-0.214) \times 0.24 \end{aligned}$$

Gene Recognition in GLIMMER

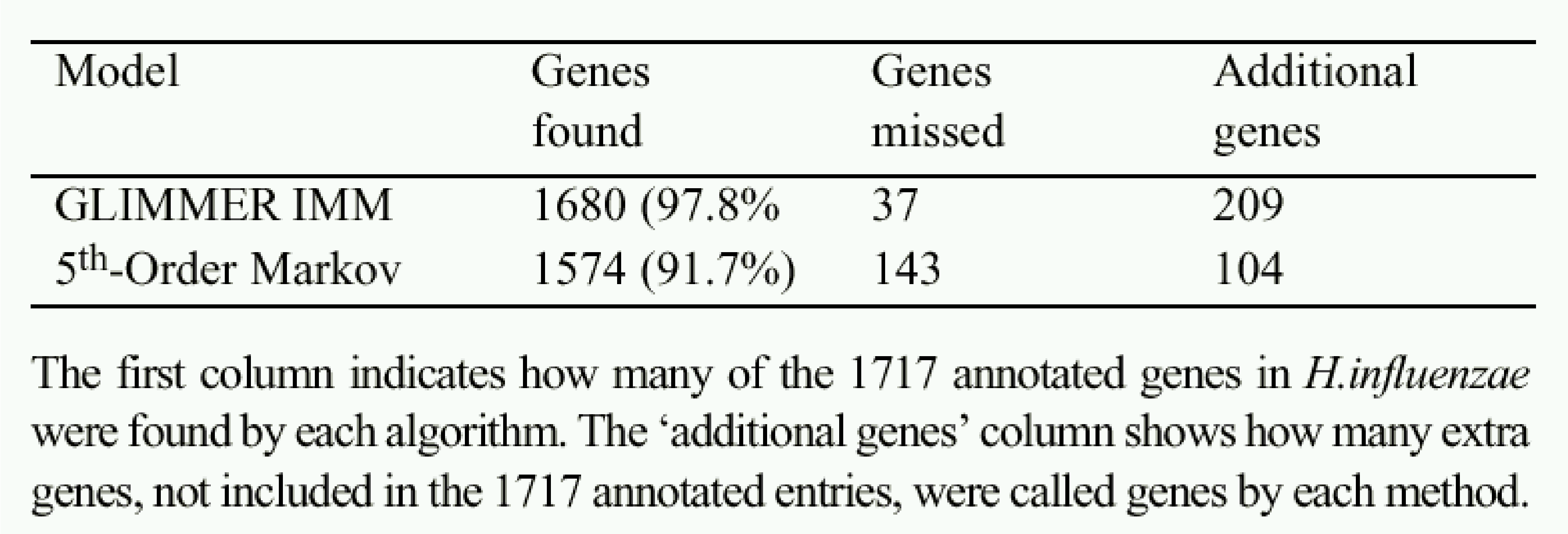
- Essentially ORF classification
- For each ORF
  - calculate the probability of the ORF sequence in each of the 6 possible reading frames
  - if the highest scoring frame corresponds to the reading frame of the ORF, mark the ORF as a gene
- For overlapping ORFs that look like genes
  - score overlapping region separately
  - predict only one of the ORFs as a gene

GLIMMER Experiment

- 8th order IMM vs. 5th order Markov model
- Trained on 1168 genes (ORFs really)
- Tested on 1717 annotated (more or less known) genes

GLIMMER Results

TP FN FP & TP?



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.

- GLIMMER has greater sensitivity than the baseline
- It's not clear if its precision/specificity is better