CMSC423: Bioinformatic Algorithms, Databases and Tools

Gene finding

Signals in DNA

- we have the genome sequence... now what?
- ...see chapter 9 ...
- Motifs are a kind of “signal” - pattern of DNA that is “unexpected” in the genome of an organism
- Uncovering new motifs – already did this – Gibbs sampling (local multiple alignment).
- Given a motif – how do we find where it occurs in a genome?
- Remember? Motif=
  - k consecutive positions
  - frequency of occurrence of each base at these positions

Finding/scoring motifs

- Given motif M of length k – can be represented as a Position Weight Matrix (PWM) – same thing as a multiple alignment profile

$$pwm_M = \{ p_{c,i} | \forall (1 \le i \le k, c \in \sigma) \}$$

- Scoring a region of the genome according to motif? Given consecutive characters $s_1,...,s_k$

$$p(M | s_{1,}.., s_k) = \prod_{1 \le i \le k} p_{s_i, i}$$

- How surprising is this? Need to compare to background probabilities

$$p(M | s_{1,}.., s_k) = \prod_{1 \le i \le k} p_{s_i, i} / q_{s_i}$$

where q_{s_i} is background probability of character s_i in genome

Scoring motifs

- Note: Score usually presented as a log-likelihood ($\log(p(M|s_1...s_k))$)
- The p/q ratios in the motif are often called Position Specific Scoring Matrix (PSSM)
- The program psi-blast can search a sequence against a database of PSSMs

- Motifs are just one piece of the puzzle
- How do we handle more complex “signals”

Gene finding/prediction

- Given a string of DNA, identify regions that might be genes
- Question: What does a gene look like?

- Start codon: ATG
- Stop codon: TGA, TAG, TAA
- Splicing: GT...intron...AG

- Also, DNA composition is different in genes – mutations are more likely in the third position of codons.

Simple gene finder (in bacteria)

- Find all stop-codons in the genome
- For each stop-codon, identify an in-frame start-codon upstream of it.
- Each section between a start and a stop is called an ORF – open reading frame.
- The long ORFs are likely genes – evolution prevented stop codons from occurring

- 3 stop codons, 64 possible codons => in random DNA every 22nd codon is a stop.

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

Gene finding as machine learning

- Main question: does the ORF look like a gene?

- Given a set of examples – genes we already know
- and a string of DNA (e.g. ORF)
- compute the likelihood that the ORF is a gene.
- Note: more complex than motif finding

- Codon usage bias – not all codons for a same amino-acid are equally likely
- K-mer (e.g. 6-mer) frequencies (instead of single-base frequencies in motif finding)

Bacillus anthracis codon usage

UUU	F	0.76	UCU	S	0.27	UAU	Y	0.77	UGU	C	0.73
UUC	F	0.24	UCC	S	0.08	UAC	Y	0.23	UGC	C	0.27
UUA	L	0.49	UCA	S	0.23	UAA	*	0.66	UGA	*	0.14
UUG	L	0.13	UCG	S	0.06	UAG	*	0.20	UGG	W	1.00
CUU	L	0.16	CCU	P	0.28	CAU	H	0.79	CGU	R	0.26
CUC	L	0.04	CCC	P	0.07	CAC	H	0.21	CGC	R	0.06
CUA	L	0.14	CCA	P	0.49	CAA	Q	0.78	CGA	R	0.16
CUG	L	0.05	CCG	P	0.16	CAG	Q	0.22	CGG	R	0.05
AUU	I	0.57	ACU	T	0.36	AAU	N	0.76	AGU	S	0.28
AUC	I	0.15	ACC	T	0.08	AAC	N	0.24	AGC	S	0.08
AUA	I	0.28	ACA	T	0.42	AAA	K	0.74	AGA	R	0.36
AUG	M	1.00	ACG	T	0.15	AAG	K	0.26	AGG	R	0.11
GUU	V	0.32	GCU	A	0.34	GAU	D	0.81	GGU	G	0.30
GUC	V	0.07	GCC	A	0.07	GAC	D	0.19	GGC	G	0.09
GUA	V	0.43	GCA	A	0.44	GAA	E	0.75	GGA	G	0.41
GUG	V	0.18	GCG	A	0.15	GAG	E	0.25	GGG	G	0.20

Simple gene finder...better

- Find ORFs
- For each ORF, compute likelihood of codon composition given codon usage table for organism:

  p(codon 1)*p(codon 2)*... * p(codon n)

- if likelihood exceeds a certain threshold – call it a gene
- note: you should calculate likelihood on equal-length windows to eliminate effect of ORF-length.

Simple gene finder: picking the threshold

- What is the right threshold?
- Answer: use training data (organism with known genes)
- Forall thresholds t between 0 and 1, stepping by 0.05
  - label all ORFs as gene/not gene
  - compare labels to known truth
- Pick threshold that makes fewest mistakes

- Actually: find best compromise between sensitivity (ability to fish out the genes) and specificity (ability to make few false predictions)

Assessing accuracy

- Confusion matrix: compare predictions to truth

truth

prediction	Gene	Not-gene
Gene	True positive	False positive Type I error
Not-gene	False negative Type II error	True negative

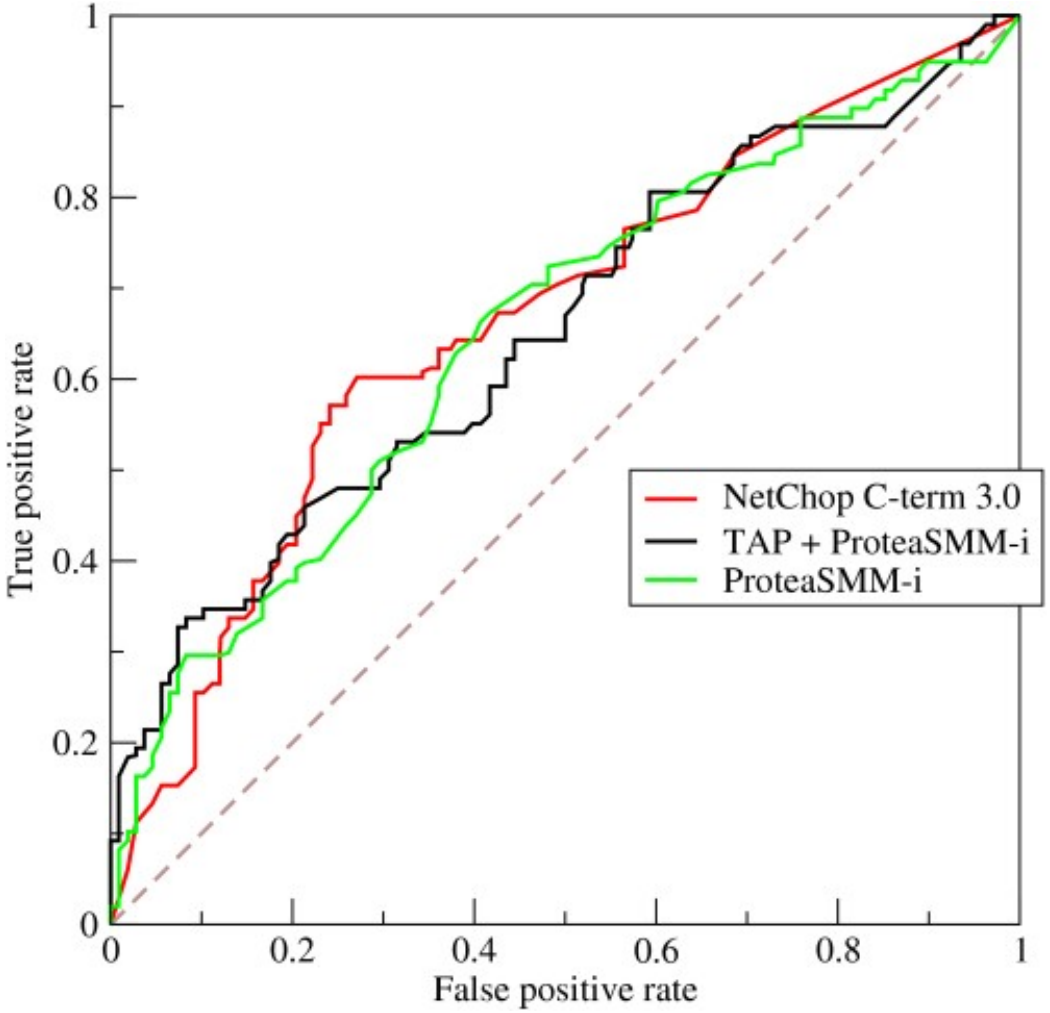
Measures of accuracy

- Sensitivity (Sn, recall) – TP/TP+FN
- Specificity (Sp) – TN/TN+FP
- Precision – TP/TP+FP

- Usually reported as (Sp, Sn), or (precision, recall).
- Also:
  F-score = 2*Precision*Recall/(Precision + Recall)

TP	FP
FN	TN

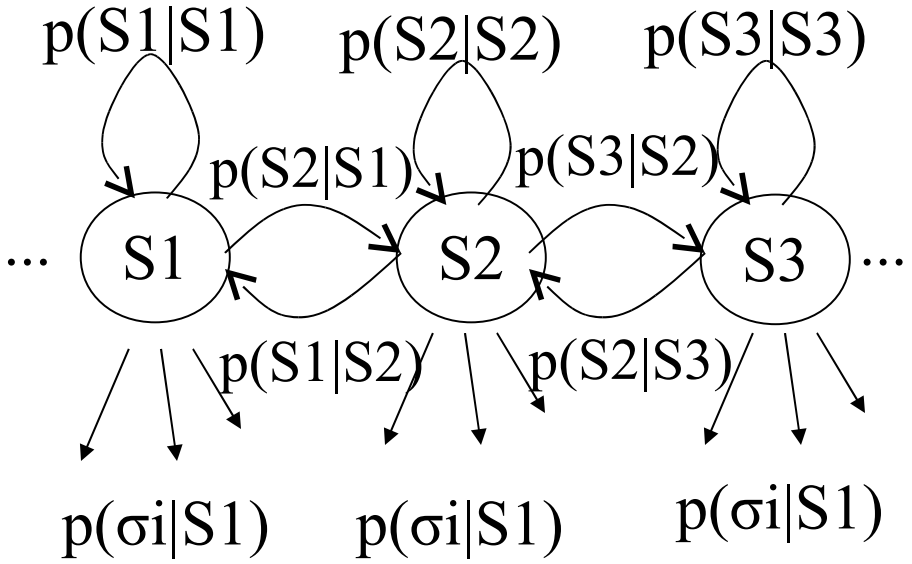
ROC(receiver operating characteristic) curve



If you vary the threshold, how do TP/FP rates change?
Higher curves are better (usually measured as Area Under ROC curve)

A more general solution

- Hidden Markov models
- States, transition probabilities, emission probabilities



- $p(S_i|S_j)$ – probability of transitioning to state i if we are in state j
- $p(\sigma_i|S_j)$ – probability of emitting symbol σ_i if we are in state j

Why “Hidden”?

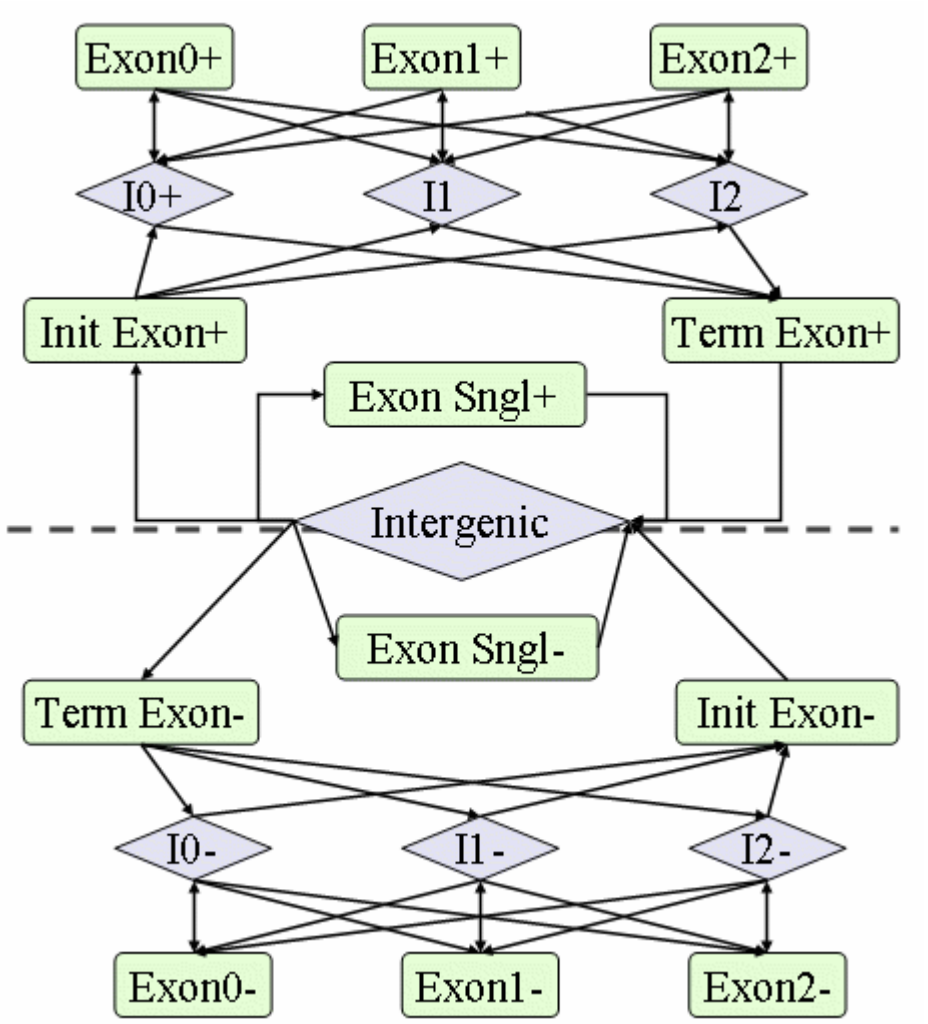
- Observers can see the emitted symbols of an HMM but have no ability to know which state the HMM is currently in.
- Thus, the goal is to infer the most likely hidden states of an HMM based on the given sequence of emitted symbols.

HMM Parameters

- Σ: set of emission characters.
  - Ex.: Σ = {H, T} for coin tossing
  - Σ = {1, 2, 3, 4, 5, 6} for dice tossing
  - Σ = {A, C, T, G} for DNA

- Q: set of hidden states, each emitting symbols from Σ.
  - Q={Fair,Biased} for coin tossing
  - Q={gene, not gene} for bacteria
  - Q={exon, intron, intergenic) for eukaryotes

GlimmerHMM model



Questions we can ask with HMMs

- Given an observed sequence of emitted characters (a string of DNA), what is the most likely sequence of states that generated the observed sequence?
  - given a string of DNA and the model, break it up into genes
  - solved by Viterbi algorithm

- Given an observed sequence of emitted characters, what is the most likely state the model was in at time t?
  - given a string of DNA, how likely is it that a certain location is inside a gene?
  - solved by forward-backward algorithm

Training – the key to HMMs

- So far we've assumed that all probabilities are known.
- The training problem:
  - given an HMM (just the states and connections)
  - given several examples (e.g. known genes and intergenic regions)
  - compute the transition and emission probabilities

- Training is difficult!!
- Baum-Welch algorithm – iterative optimization
  - start with estimates of the probabilities
  - run model with training data
  - re-estimate probabilities based on performance on training data

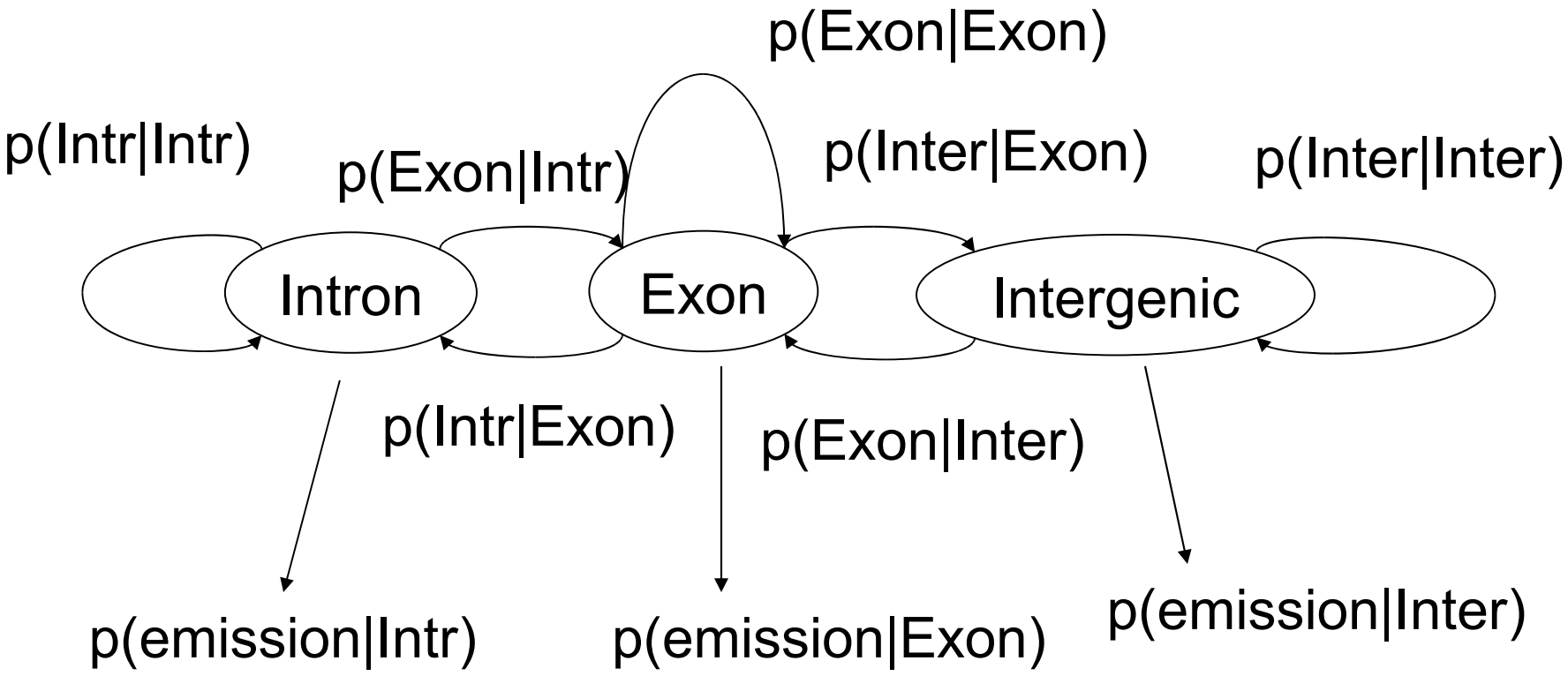
Questions

- Given the G/C content for a genome (fraction of letters in the genome that are G or C), what is the expected distance between two stop codons? - requires Poisson statistics

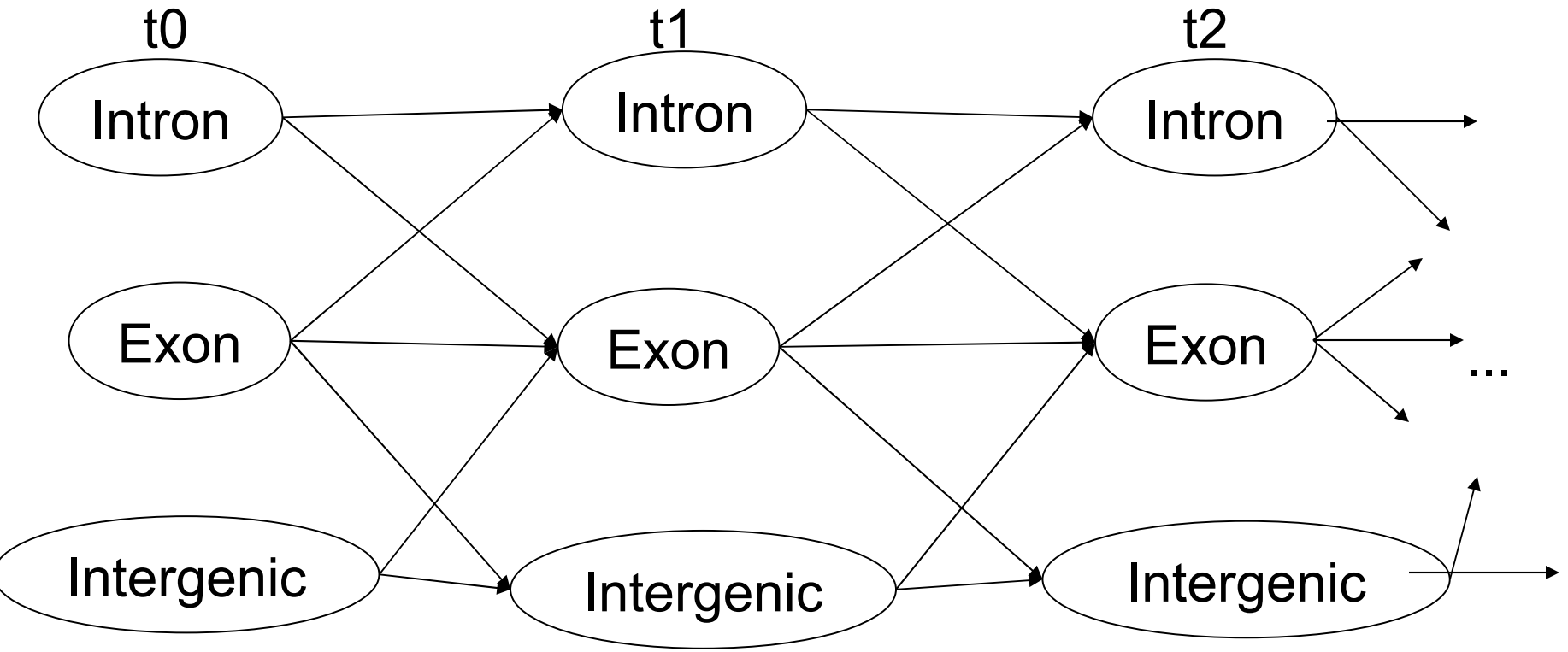
Advanced material (not on exam)

Viterbi algorithm

- Given an HMM and an output string, compute the most likely path through the HMM that would result in the given string



Viterbi algorithm



Observations:

x0 x1 x2

maximize $\prod_{0}^{n} e_{statej}(x_j)\, p(state_j \mid state_{j-1})$ over all possible state paths

dynamic programming algorithm

Viterbi algorithm

- S(k,i) – most likely path for $x_0..x_i$ ends in state k
- $S(l, i + 1) = \max_k \{ S(k, i) * p(l|k) * p(\text{emission of } x_{i+1}|l)\}$
  $= p(\text{emission of } x_{i+1}|l) * \max_k \{S(k,i) * p(l|k)\}$

- The optimal path is found by back-tracking
- Note: Viterbi is equivalent to finding longest path in a graph
- Implementation problem: underflow – many products of very small values
- Solution: work in log-space
  - instead of probabilities use logarithm of probabilities
  - instead of products use sums

Forward-backward algorithm

- Given an HMM and an output string of length n, what is the probability that the HMM was in state k at time i < n?

- Similar dynamic programming as Viterbi however done twice:
  - from t0 to ti (forwards)
  - from tn to ti (backwards)
- In Viterbi recurrence replace max with $\sum$
  - likelihood is a sum of probabilities - all possible paths that go through state k at time i

Notes on training an HMM

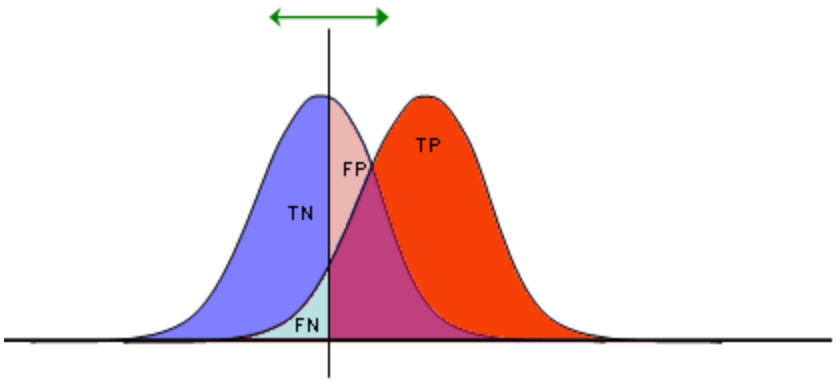
- Gene finder output
  - a set of predictions (exon, intron, intergenic, etc.)
  - a probability (likelihood) for each prediction
- In addition to setting parameters for the model you also need to pick a threshold – how high should the probability be before you "believe" it.

Picking the "right" threshold

- Cross-validation (hold-out cross validation)
  - divide training set into Training and Hold sets
  - train in "Training"
  - test result on "Hold" – adjust threshold until results look best

- k-fold cross-validation
  - divide training set into K sub-sets
  - train on K-1 sets and test on one of them
  - repeat for different choices of "test" set

Receiver operating characteristic



TP	FP
FN	TN
1	1