Gene Prediction

Introduction

- Gene: A sequence of nucleotides coding for protein
- Gene Prediction Problem: Determine the beginning and end positions of genes in a genome

-
```
atgcatgcggctatgctaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcggctatgct
aatgcatgcggctatgcaagctgggatccgatgactatgctaagctgggatccgatgacaatgcatgcggc
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ctatgctaatgcatgcggctatgcaagctgggatccgatgactatgctaagctgcggctatgctaatgcat
gcggctatgctaagct
```

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- atgcatgcggctatgctaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcggctatgct
  aatgcatgcggctatgcaagctgggatccgatgactatgctaagctgggatccgatgacaatgcatgcggc
  tatgctaatgaatggtcttgggatttaccttggaatgctaagctgggatccgatgacaatgcatgcggcta
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  gctatgctaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcggctatgctaatgcatgc
  ggctatgcaagctgggatcctgcggctatgctaatgaatggtcttgggatttaccttggaatgctaagctg
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  tgcggctatgctaagctgggaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcggctat
  gctaatgcatgcggctatgcaagctgggatccgatgactatgctaagctgcggctatgctaatgcatgcgg
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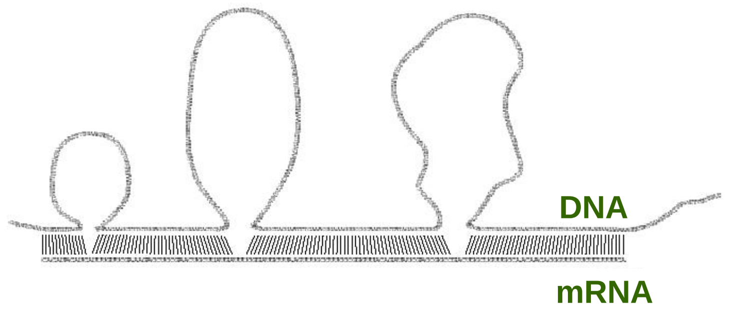
  gctgggatccgatgacaatgcatgcggctatgctaatgaatggtcttgggatttaccttggaatatgctaa
  tgcatgcggctatgctaagctgggaatgcatgcggctatgctaagctgggatccgatgacaatgcatgcgg
  ctatgctaatgcatgcggctatgcaagctgggatccgatgactatgctaagctgcggctatgctaatgcat
  gcggctatgctaagct

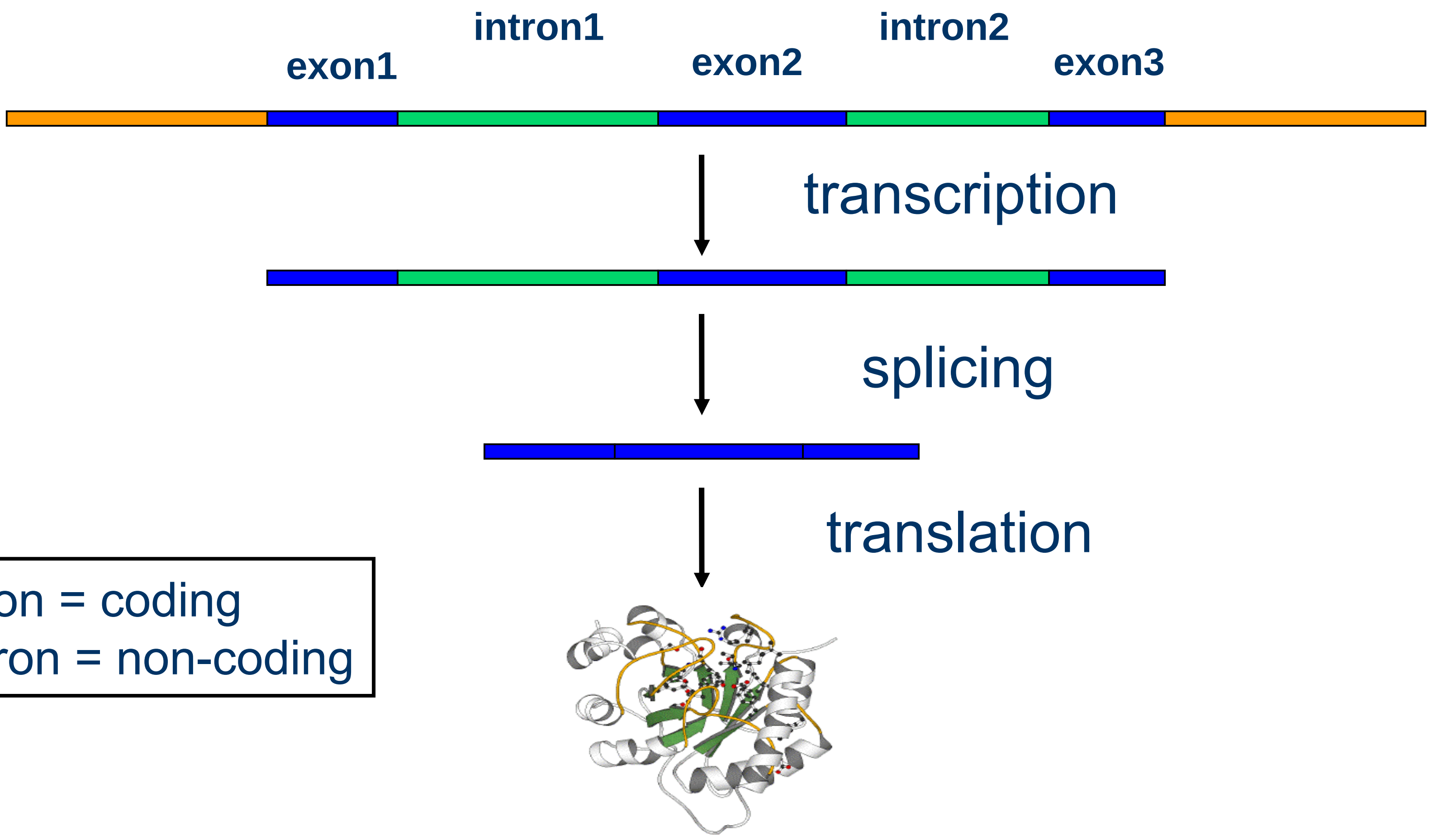
Gene!

Introduction

- In 1960's it was discovered that the sequence of codons in a gene determines the sequence of amino acids in a protein
  - ➢ an incorrect assumption: the triplets encoding for amino acid sequences form contiguous strips of information.
- A paradox: genome size of many eukaryotes does not correspond to "genetic complexity", for example, the salamander genome is 10 times the size of that of human.
- 1977 – discovery of "split" genes: experiments with mRNA of *hexon*, a viral protein:
  - mRNA-DNA hybrids formed three curious loop structures instead of contiguous duplex segments (seen in an electron microscope)



Central Dogma and Splicing



exon = coding
intron = non-coding

Gene prediction is hard

- The Genome of many eukaryotes contain only relatively few genes (Human genome 3%).
- Many false splice sites & other signals.
- Very short exons (3bp), especially initial.
- Many very long introns.
- Alternative splicing

Approaches to gene finding

A. Statistical or ab initio methods: These methods attempt to predict genes based on statistical properties of the given DNA sequence. Programs are e.g. Genscan, GeneID, GENIE and FGENEH.

B. Comparative methods: The given DNA string is compared with a similar DNA string from a different species at the appropriate evolutionary distance and genes are predicted in both sequences based on the assumption that exons will be well conserved, whereas introns will not. Programs are e.g. CEM (conserved exon method) and Twinscan.

C. Homology methods: The given DNA sequence is compared with known protein structures. Programs are e.g. TBLASTN or TBLASTX, Procrustes and GeneWise.

A. Statistical ab initio methods

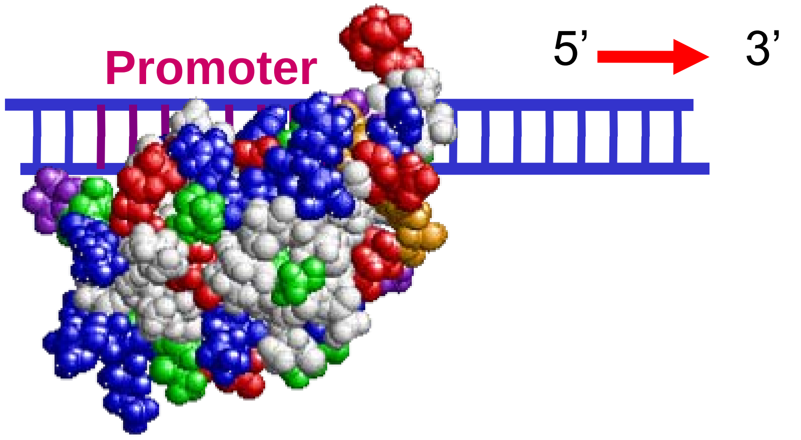
- Coding segments (exons) have typical sequences on either end and use different subwords than non-coding segments (introns).
- E.g. for the bases around the transcription start site we may have the following observed frequencies (given by this position specific weight matrix (PSWM)):

Pos.	-8	-7	-6	-5	-4	-3	-2	-1	+1	+2	+3	+4	+5	+6	+7
A	.16	.29	.20	.25	.22	.66	.27	.15	1	0	0	.28	.24	.11	.26
C	.48	.31	.21	.33	.56	.05	.50	.58	0	0	0	.16	.29	.24	.40
G	.18	.16	.46	.21	.17	.27	.12	.22	0	0	1	.48	.20	.45	.21
T	.19	.24	.14	.21	.06	.02	.11	.05	0	1	0	.09	.26	.21	.21

- This can then be used together in a log-likelihood scoring model in order to distinguish certain recognition sites (such as transcription start sites, or promoter regions) from non-recognition sites.

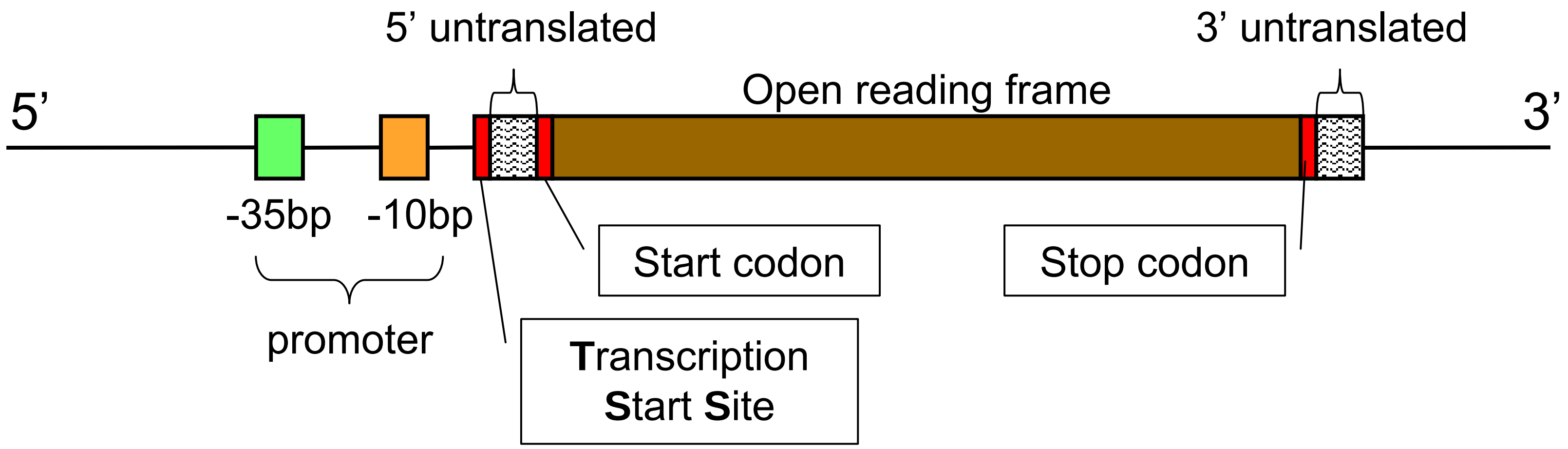
A. Gene prediction in prokaryotes
gene structure

- Most DNA is coding
- No introns
- Promoters are DNA segments upstream of transcripts that initiate transcription



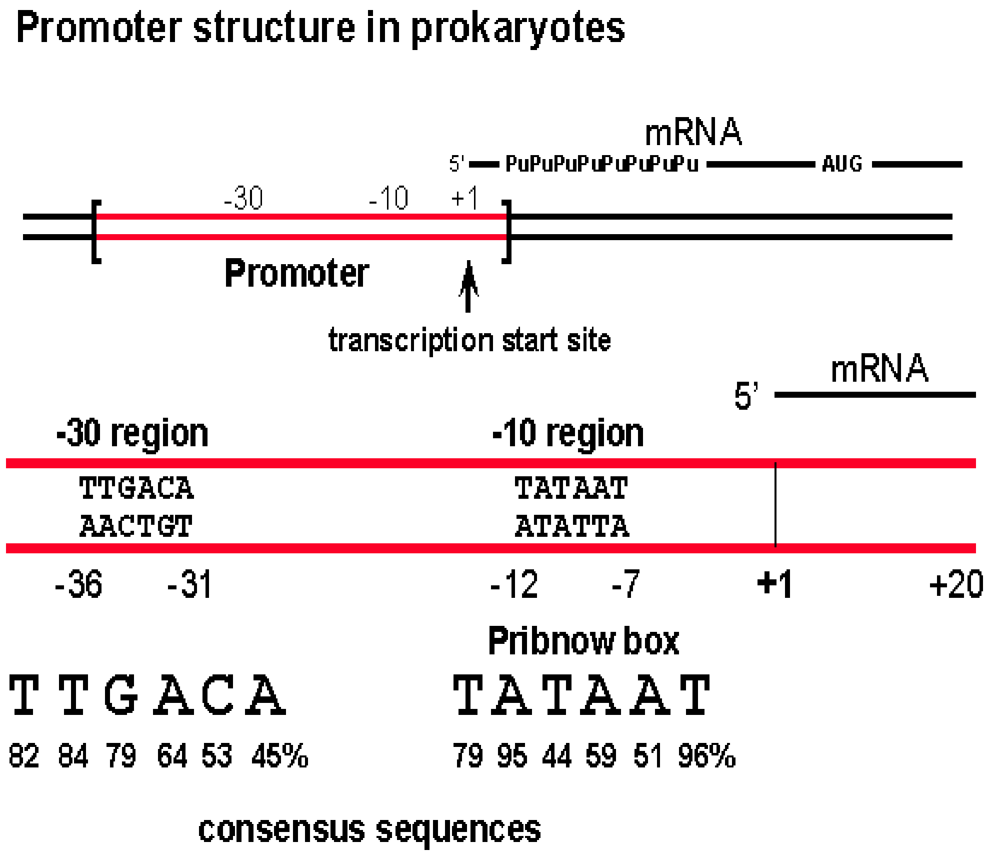
- Promoter *attracts* RNA Polymerase to the transcription start site

A. Gene prediction in prokaryotes
gene structure



- Upstream transcription start site (TSS; position 0) there are promoters

Promoter Structure in Prokaryotes (E.Coli)

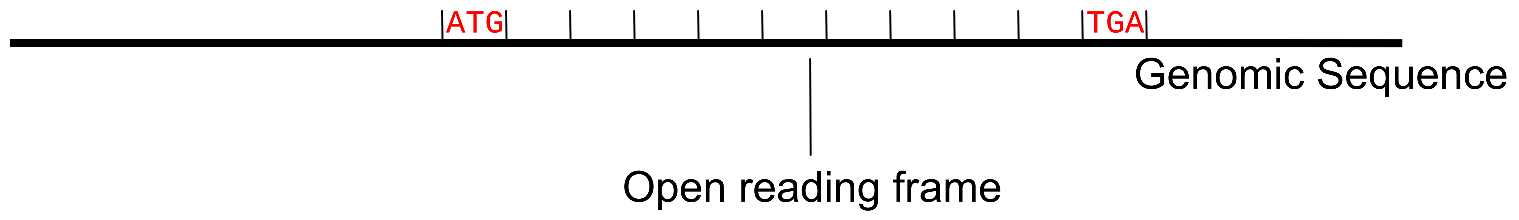


Transcription starts at offset 0.

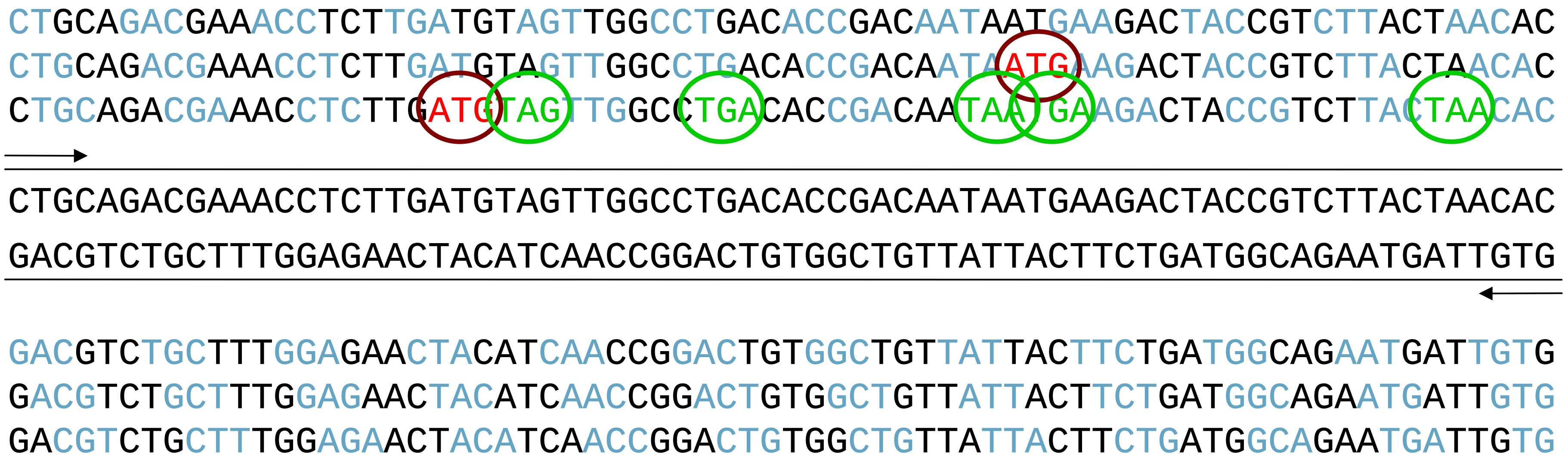
- Pribnow Box (-10)
- Gilbert Box (-30)
- Ribosomal Binding Site (+10)

Open Reading Frames (ORFs)

- Detect potential coding regions by looking at ORFs
  - A genome of length *n* is comprised of (*n*/3) codons
  - Stop codons (TAA, TAG or TGA) break genome into segments between consecutive Stop codons
  - The subsegments of these that start from the Start codon (ATG) are ORFs
    - ORFs in different frames may overlap



Six Frames in a DNA Sequence



```
CTGCAGACGAAACCTCTTGATGTAGTTGGCCTGACACCGACAATAATGAAGACTACCGTCTTACTAACAC
CTGCAGACGAAACCTCTTGATGTAGTTGGCCTGACACCGACAATAATGAAGACTACCGTCTTACTAACAC
CTGCAGACGAAACCTCTTGATGTAGTTGGCCTGACACCGACAATAATGAAGACTACCGTCTTACTAACAC

CTGCAGACGAAACCTCTTGATGTAGTTGGCCTGACACCGACAATAATGAAGACTACCGTCTTACTAACAC
GACGTCTGCTTTGGAGAACTACATCAACCGGACTGTGGCTGTTATTACTTCTGATGGCAGAATGATTGTG

GACGTCTGCTTTGGAGAACTACATCAACCGGACTGTGGCTGTTATTACTTCTGATGGCAGAATGATTGTG
GACGTCTGCTTTGGAGAACTACATCAACCGGACTGTGGCTGTTATTACTTCTGATGGCAGAATGATTGTG
GACGTCTGCTTTGGAGAACTACATCAACCGGACTGTGGCTGTTATTACTTCTGATGGCAGAATGATTGTG
```

- stop codons – `TAA, TAG, TGA`
- start codons – `ATG`

ORF prediction

1. **Evaluation of ORF length**
   - In an “random” DNA, the average distance between stop codons is 64/3 ≈ 21 which is much less than the average length of a protein (≈300)
   - Simple algorithm, poor performance
2. **Evaluation of codon usage**
   - Codon usage in coding regions differs form the codon usage in non-coding regions
3. **Evaluation of codon preference**
   - One aminoacid is coded by several different codons, some of them are used more often than the others (see below)
4. **Markov models and HMMs**

2. ORF prediction – codon usage

I. Codon usage (see below)
- Create a 64-element hash table and count the frequencies of codons in an ORF
- Uneven use of the codons may characterize a real gene
- This compensate for pitfalls of the ORF length test

II. Hexamer counts
- Frequency of occurrences of oligonucleotides of length 6 in a reading frame
- Usually modeled as fifth-order Hidden Markov Models

$$P(x_n = s \mid \cap_{j<n} x_j) = P(x_n = s \mid x_{n-1}x_{n-2}x_{n-3}x_{n-4}x_{n-5})$$

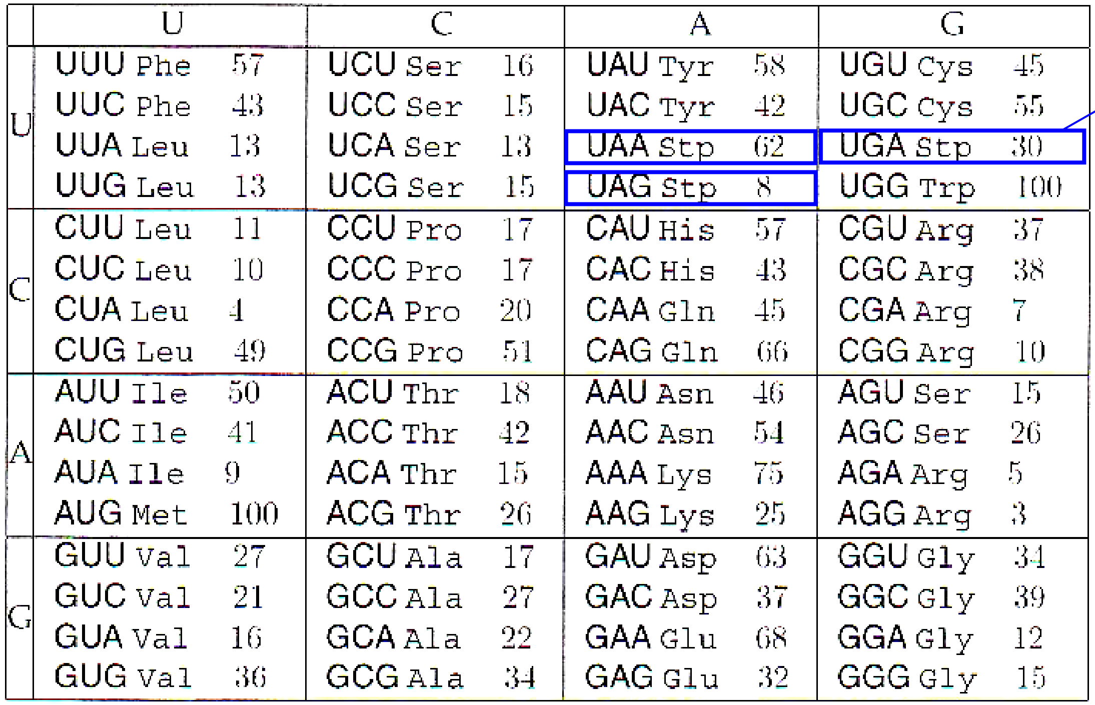
2. ORF prediction – codon usage

- Vector with 64 components – frequencies of usage for each codon

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

Codon Usage in Human Genome

- Amino acids typically have more than one codon, but in nature certain codons are more in use



	U	C	A	G
U	UUU Phe 57	UCU Ser 16	UAU Tyr 58	UGU Cys 45
	UUC Phe 43	UCC Ser 15	UAC Tyr 42	UGC Cys 55
	UUA Leu 13	UCA Ser 13	UAA Stp 62	UGA Stp 30
	UUG Leu 13	UCG Ser 15	UAG Stp 8	UGG Trp 100
C	CUU Leu 11	CCU Pro 17	CAU His 57	CGU Arg 37
	CUC Leu 10	CCC Pro 17	CAC His 43	CGC Arg 38
	CUA Leu 4	CCA Pro 20	CAA Gln 45	CGA Arg 7
	CUG Leu 49	CCG Pro 51	CAG Gln 66	CGG Arg 10
A	AUU Ile 50	ACU Thr 18	AAU Asn 46	AGU Ser 15
	AUC Ile 41	ACC Thr 42	AAC Asn 54	AGC Ser 26
	AUA Ile 9	ACA Thr 15	AAA Lys 75	AGA Arg 5
	AUG Met 100	ACG Thr 26	AAG Lys 25	AGG Arg 3
G	GUU Val 27	GCU Ala 17	GAU Asp 63	GGU Gly 34
	GUC Val 21	GCC Ala 27	GAC Asp 37	GGC Gly 39
	GUA Val 16	GCA Ala 22	GAA Glu 68	GGA Gly 12
	GUG Val 36	GCG Ala 34	GAG Glu 32	GGG Gly 15

Percents of usage among all codons encoding Stp

Codon Usage in Mouse Genome

AA	codon	/1000	frac
Ser	TCG	4.31	0.05
Ser	TCA	11.44	0.14
Ser	TCT	15.70	0.19
Ser	TCC	17.92	0.22
Ser	AGT	12.25	0.15
Ser	AGC	19.54	0.24
Pro	CCG	6.33	0.11
Pro	CCA	17.10	0.28
Pro	CCT	18.31	0.30
Pro	CCC	18.42	0.31

AA	codon	/1000	frac
Leu	CTG	39.95	0.49
Leu	CTA	7.89	0.10
Leu	CTT	12.97	0.16
Leu	CTC	20.04	0.25
Ala	GCG	6.72	0.10
Ala	GCA	15.80	0.23
Ala	GCT	20.12	0.29
Ala	GCC	26.51	0.38
Gln	CAG	34.18	0.75
Gln	CAA	11.51	0.25

3. ORF prediction – codon preference

- For each reading frame a codon preference statistics at each position is computed. The statistic is calculated over a window of length l_w (l_w is usually between 25 and 50), where the window is moved along the sequence in increments of three bases, maintaining the reading frame. The magnitude of the codon preference statistic is a measure of the likeness of a particular window of codons to a predetermined preferred usage.
- The statistic is based on the concept of **synonymous codons**. Synonymous codons are those codons specifying the same amino acid.

3. ORF prediction – codon preference

- Example:

  Leucine, Alanine and Tryptophan are coded by 6, 4 and 1 different codons respectively. Hence in a uniformly random DNA they should occur in the ratio 6:4:1. But in a protein they occur in the ratio 6.9:6.5:1.

- For a codon c

f_c	codon's frequency of occurrence in the window.
F_c	the total number of occurrences of c's synonymous family in the window.
r_c	the calculated number of occurrences of c in a random sequence of length l_w with the same base composition as the sequence being analyzed.
R_c	the calculated number of occurrences of c's synonymous family in a random sequence of length l_w with the same base composition as the sequence being analyzed.

3. ORF prediction – codon preference

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R_c	the calculated number of occurrences of c's synonymous family in a random sequence of length l_w with the same base composition as the sequence being analyzed.

- The codon *c* preference statistic:

$$p_c = \frac{f_c / F_c}{r_c / R_c}$$

  – if $p_c = 1$ *c* is used equally in a random sequence and in the codon frequency table

3. ORF prediction – codon preference

- When an aminoacid is coded by codons $c_1, c_2, \ldots, c_k$, then obviously

$$\prod_{j=1}^{k} p_{c_j} \le 1$$

- The probability of the sequence in the window w is then $P(w) = \prod_{i=1}^{l_w} p_{c_j}$

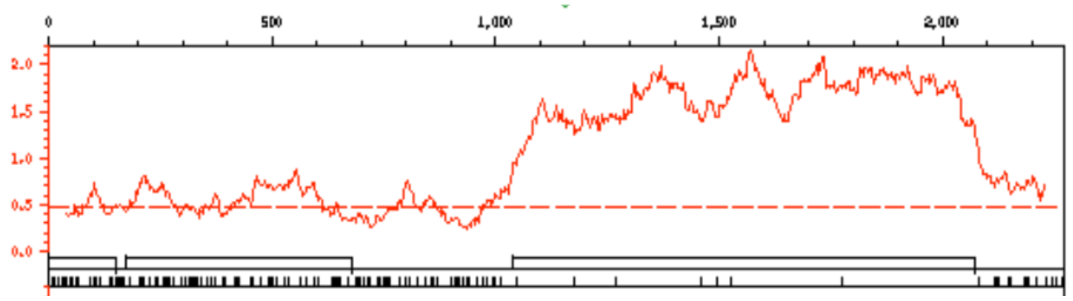
- Log-based score is used instead and the codon preference statistics for each window is

$$P_w = e^{\frac{\sum_{i=1}^{l_w} \log p_{c_i}}{l_w}}$$

- A correction: 0 in the

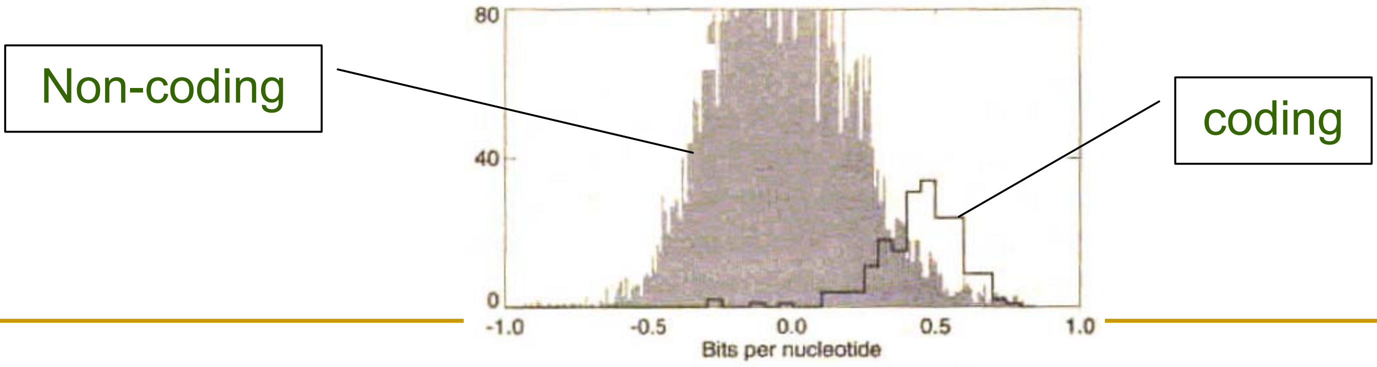
  codon frequency table

  is replaced by $1/F_c$



4. ORF prediction – using Markov models and HMM

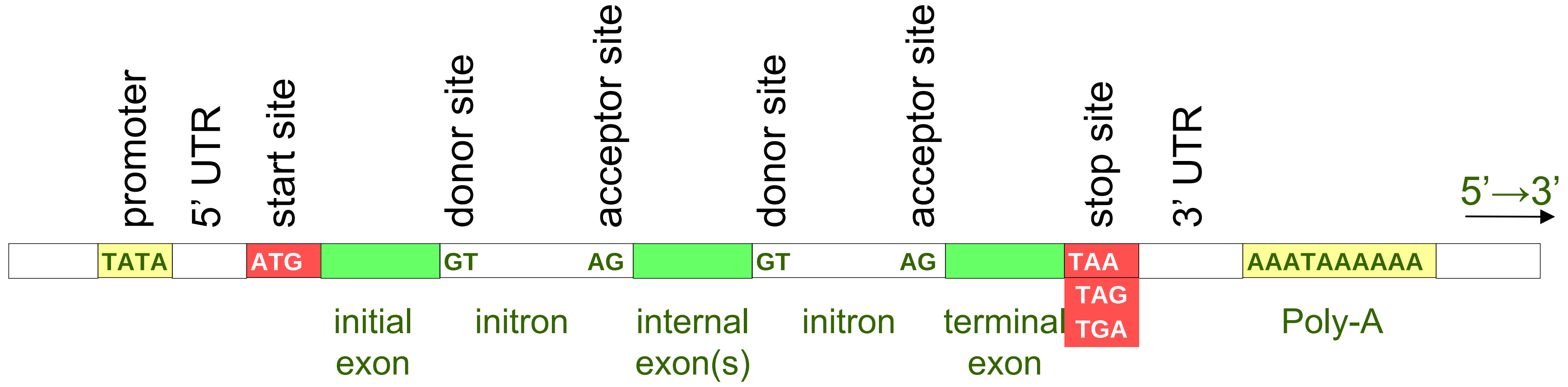
- There are many more ORFs than real genes. E.g., the E. coli genome contains about 6600 ORFs but only about 4400 real genes. Markov model and an HMM can be used to distinguish between non-coding ORFs and real genes.
- DNA can be modeled as 64-state Markov chain of codons:
  - Probabilities that a certain codon is followed by another one in a coding ORF is computed.
  - Probability of the chain is then computed in the form of log-odds score.
  - Non-coding ORF has log-odds distribution centered around 0.



A. Gene prediction in eukaryotes

gene structure

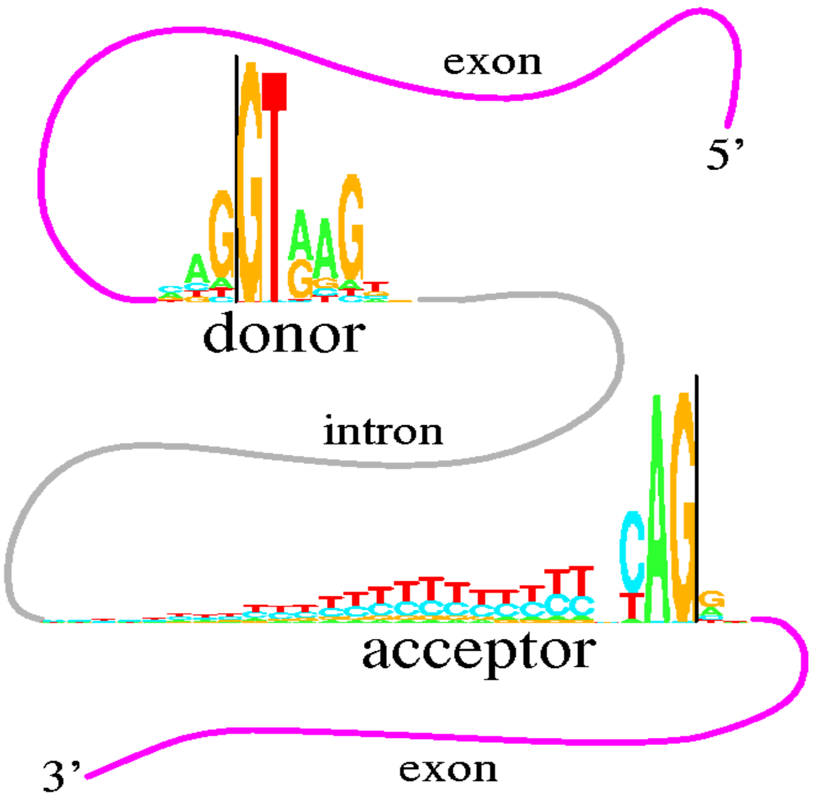
- Alternating exons and introns



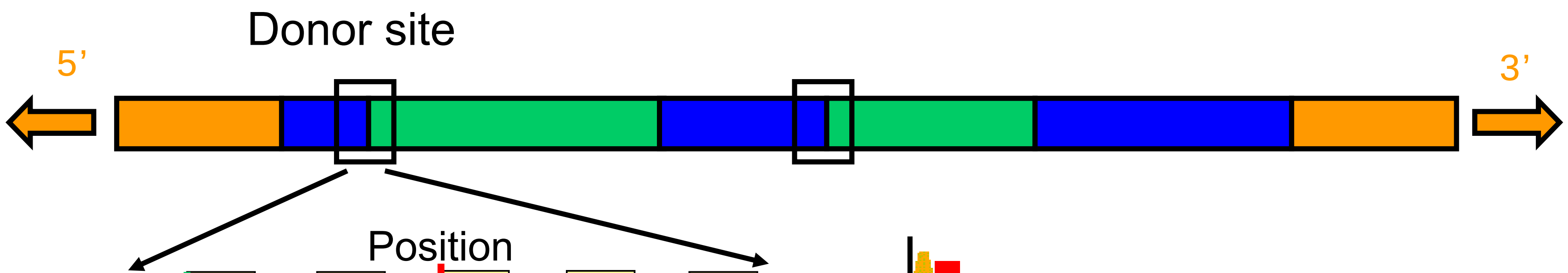
- intron starts usually by AG and ends by GT
- Types of exons
  1. Initial exons
  2. Internal exons
  3. Terminal exons
  4. Single-exon genes, i.e. genes without introns.

Consensus splice sites – splicing signals

- Try to recognize location of splicing signals at exon-intron junctions
  - This has yielded a weakly conserved donor splice site and acceptor splice site
- Profiles for sites are still weak, and lends the problem to the Hidden Markov Model (HMM) approaches, which capture the statistical dependencies between sites

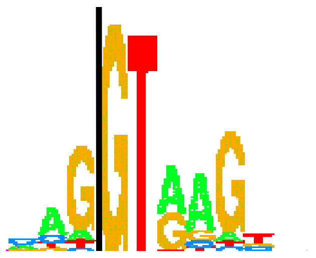


Splice site detection



Position

%	-8	...	-2	-1	0	1	2	...	17
A	26	...	60	9	0	1	54	...	21
C	26	...	15	5	0	1	2	...	27
G	25	...	12	78	99	0	41	...	27
T	23	...	13	8	1	98	3	...	25

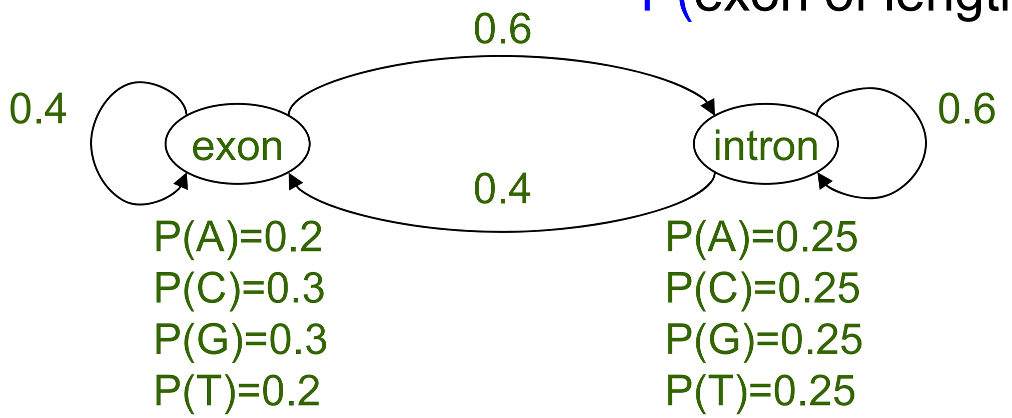


- In the exon-intron junctions there is a large similarity to the consensus sequence → algorithms based on position specific weight matrices.
- However, this is far too simple, since it does not use all the information encoded in a gene. Thus more integrated approaches are sought. This naturally leads us to Hidden Markov Models.

A simple HMM M for gene detection

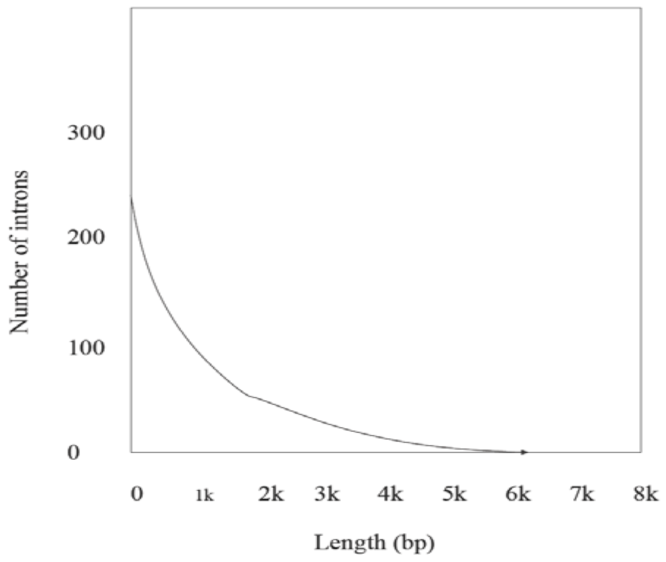
- States are ‘in exon’ and ‘in intron’
- p probability that the process stays ‘in exon’; $1–p$ probability that the process switches into ‘in intron’
- q probability that the process stays ‘in intron’; $1–q$ probability that the process switches into ‘in exon’
- The probability that an exon has length k is

$$P(\text{exon of length } k \mid M) = p^k (1–p)$$

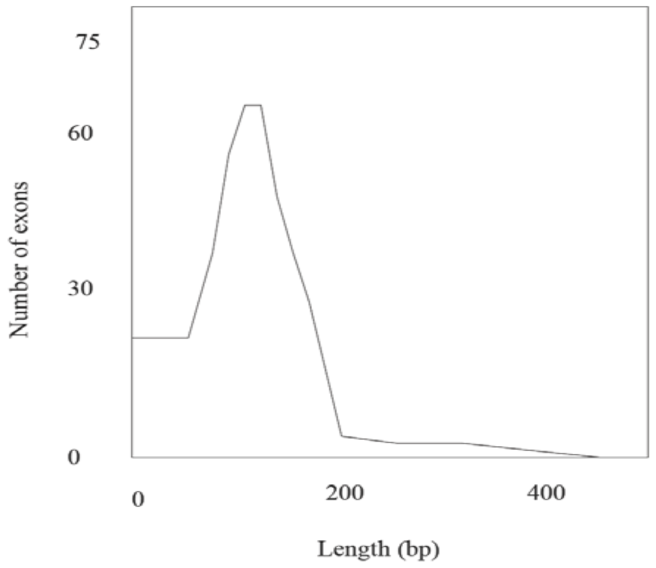


A simple HMM M for gene detection

- $p^k (1-p)$ implies geometric distribution which does not correspond to the real distribution of lengths of introns and exons



introns



initial exons

A simple HMM M for gene detection

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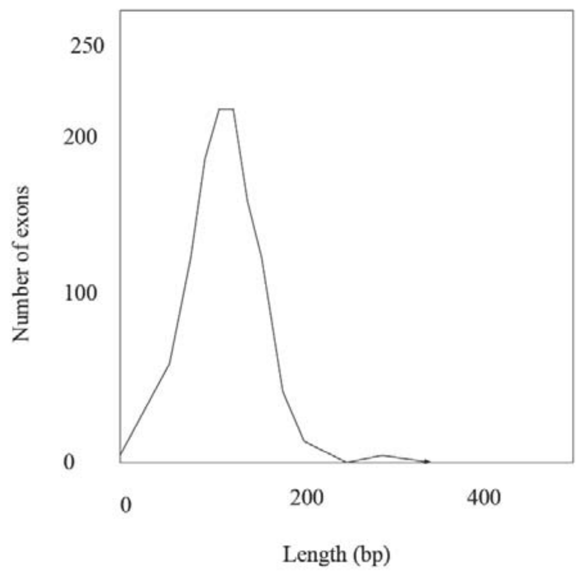
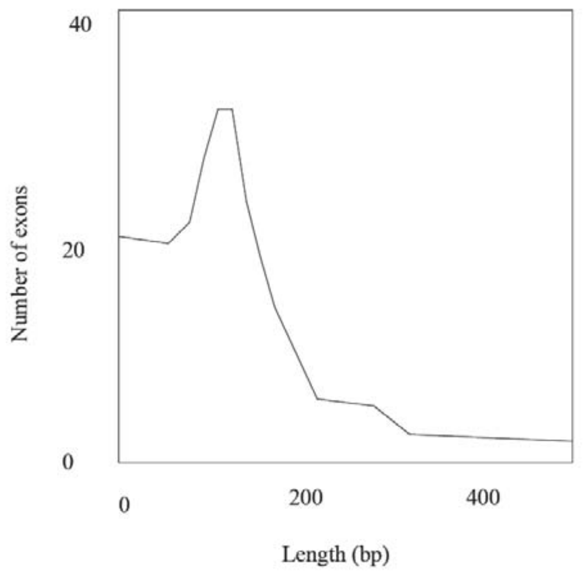




internal exons

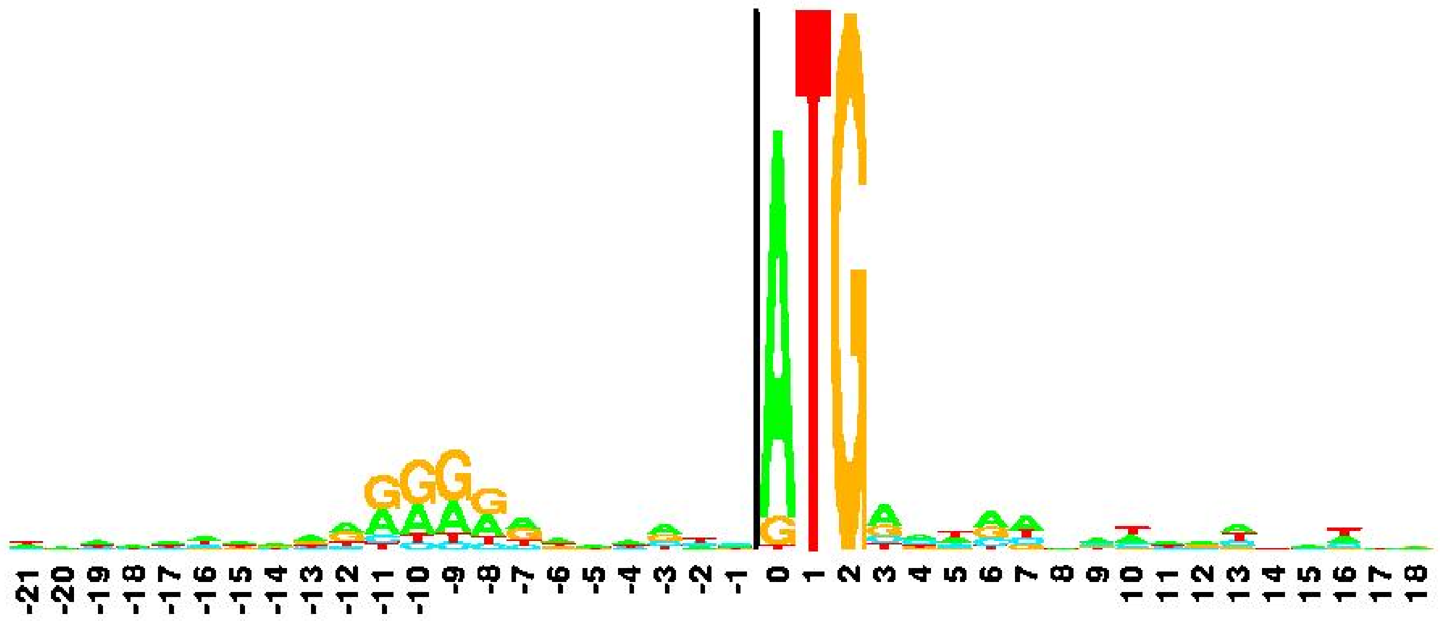
terminal exons

A simple HMM M for gene detection

- If an exon is too short (under 50bp), the spliceosome (enzyme that performs the splicing) has not enough room.
- Exons that are longer than 300 bp are difficult to locate.
- Typical numbers for vertebrates:
  - mean gene length ≈ 30kb,
  - mean coding region length ≈ 1−2kb.
- **we need other models that can model biological exon lengths**

Ribosomal Binding Site

1055 E. coli Ribosome binding sites listed in the Miller book



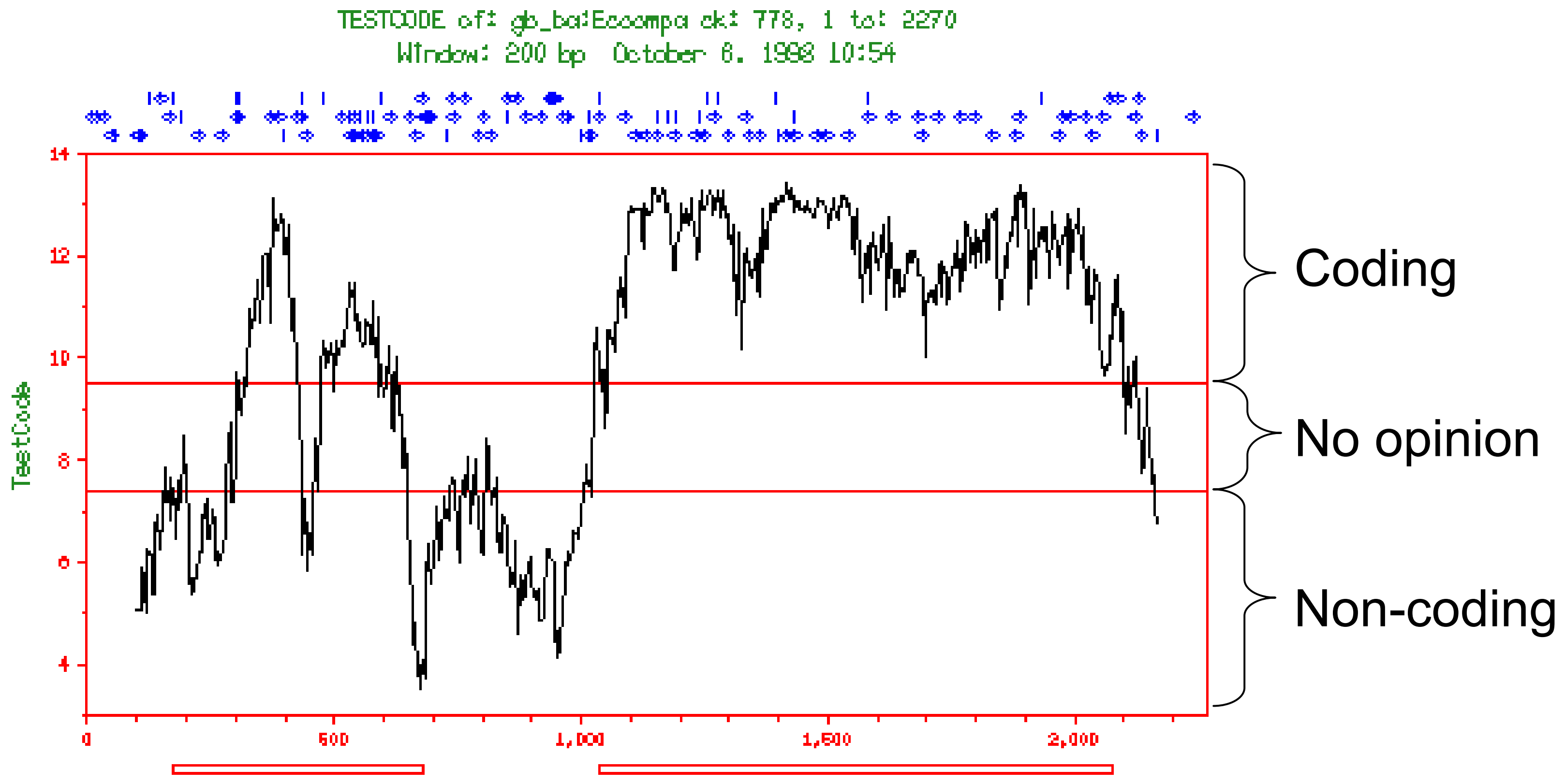
TestCode

- Statistical test described by James Fickett in 1982: tendency for nucleotides in coding regions to be repeated with periodicity of 3
  - Judges randomness instead of codon frequency.
  - Finds “putative” coding regions, not introns, exons, or splice sites.
- TestCode finds ORFs based on compositional bias with a periodicity of three.

TestCode Statistics

- Define a window size no less than 200 bp, slide the window along the sequence down 3 bases. In each window:
  - Calculate for each base {A, T, G, C}
    - max (n_{3k+1}, n_{3k+2}, n_{3k}) / min (n_{3k+1}, n_{3k+2}, n_{3k})
    - Use these values to obtain a probability from a lookup table (which was a previously defined and determined experimentally with known coding and noncoding sequences
- Probabilities can be classified as indicative of " coding” or “noncoding” regions, or “no opinion” when it is unclear what level of randomization tolerance a sequence carries
- The resulting sequence of probabilities can be plotted

TestCode Sample Output



Popular Gene Prediction Algorithms

- **GENSCAN**: uses modified Hidden Markov Models (HMMs) – semi-Markov model – based on statistical methods and on data from an annotated training set

- **TWINSCAN**
  - Uses both HMM and similarity (e.g., between human and mouse genomes)

B. Comparative gene finding

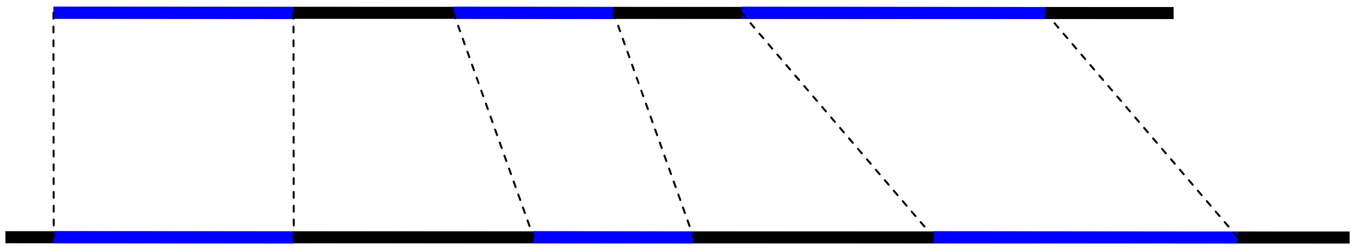
- **Idea**: the level of sequence conservation between two species depends on the function of the DNA, e.g. coding sequence is more conserved than intergenic sequence.
- Program Rosetta:
  - first computes a global alignment of two homologous sequences
  - and then attempts to predict genes in both sequences simultaneously.
- A conserved exon method: that uses local conservation.
- *Orthologous Genes*: homologous genes in two species that have a common ancestor.

Using Known Genes to Predict New Genes

- Some genomes may be very well-studied, with many genes having been experimentally verified.
- Closely-related organisms may have similar genes.
- Unknown genes in one species may be compared to genes in some closely-related species.
- Most human genes have mouse orthologs:
  - 95% of coding exons are in a one-to-one correspondence between the two genomes.
  - 75% of orthologous coding exons have equal length, and
  - 95% have equal length modulo 3.
  - Intron lengths differ by an average of 50%.
  - The coding sequence similarity between the two organisms is around 85%,
  - the intron sequence similarity is around 35%,
  - 5’ UTRs and 3’ UTRs around 68%.

Similarity-Based Approach to Gene Prediction

- Genes in different organisms are similar
- The similarity-based approach uses known genes in one genome to predict (unknown) genes in another genome
- **Problem:** Given a known gene and an unannotated genome sequence, find a set of substrings of the genomic sequence whose concatenation best fits the gene

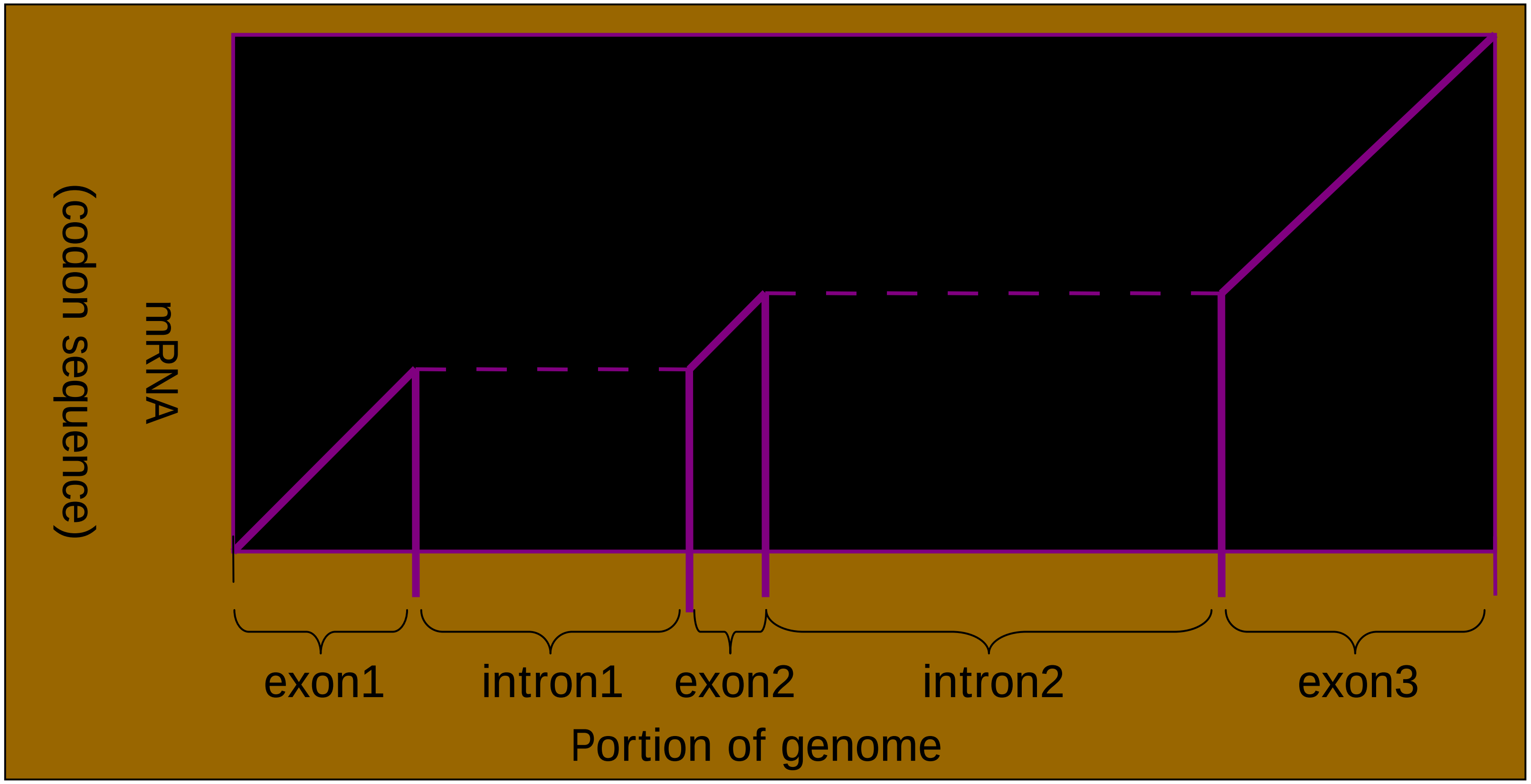


- We try to identify small islands of similarity corresponding to similarities between exons

Reverse Translation

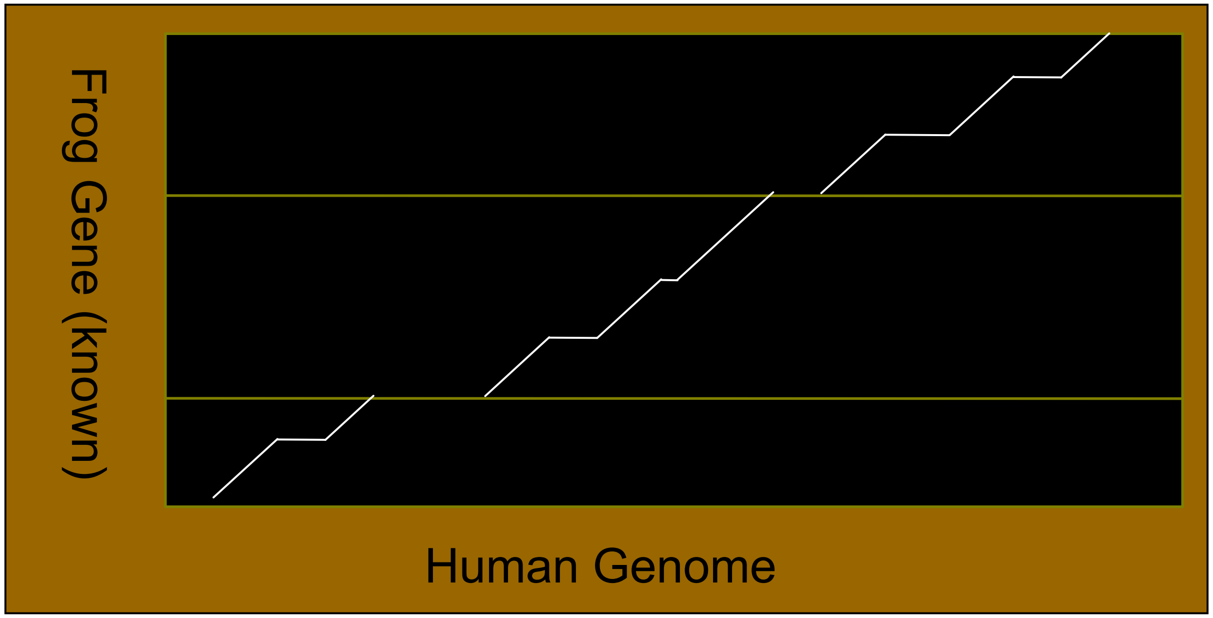
- Given a known protein, find a gene in the genome which codes for it.
- One might infer the coding DNA of the given protein by reversing the translation process
  - Inexact: amino acids map to > 1 codon.
  - This problem is essentially reduced to an alignment problem.
- This reverse translation problem can be modeled as traveling in Manhattan grid with free horizontal jumps
  - Complexity of Manhattan is n^3
- Every horizontal jump models an insertion of an intron
- Problem with this approach: would match nucleotides pointwise and use horizontal jumps at every opportunity

Comparing Genomic DNA Against mRNA



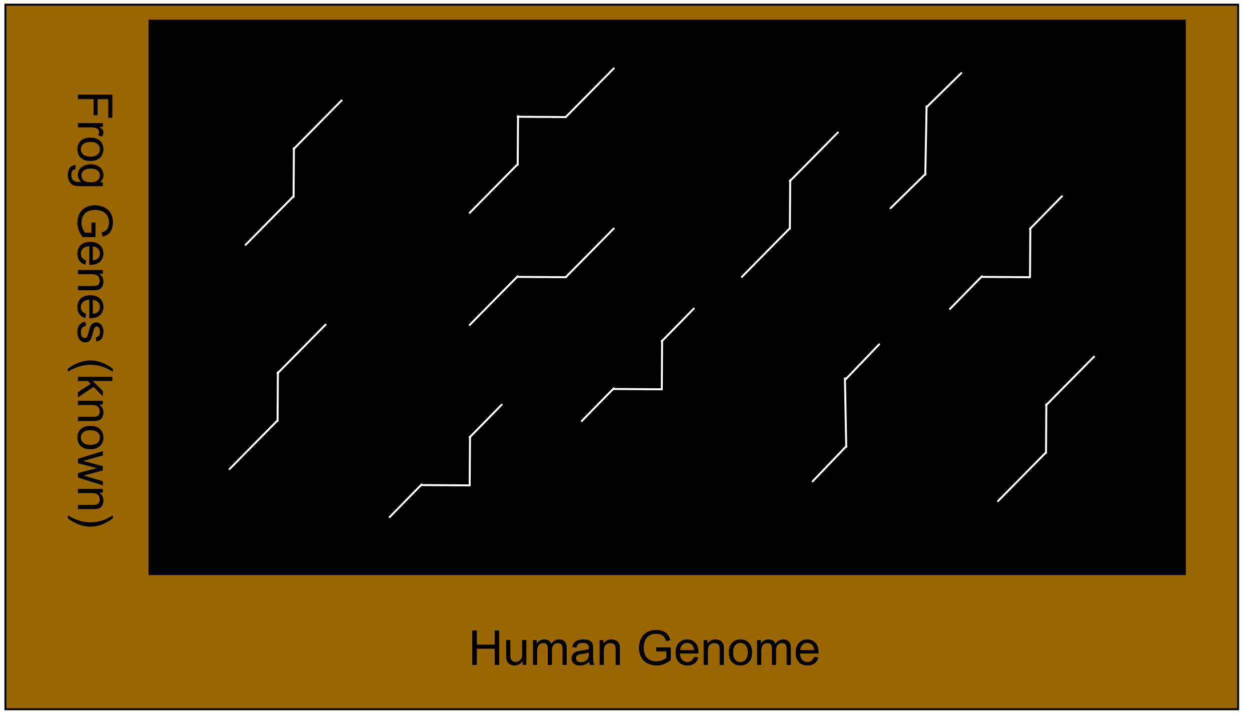
Using Similarities to Find the Exon Structure

- The known frog gene is aligned to different locations in the human genome
- Find the “best” path to reveal the exon structure of human gene



Finding Local Alignments

- Use local alignments to find all islands of similarity



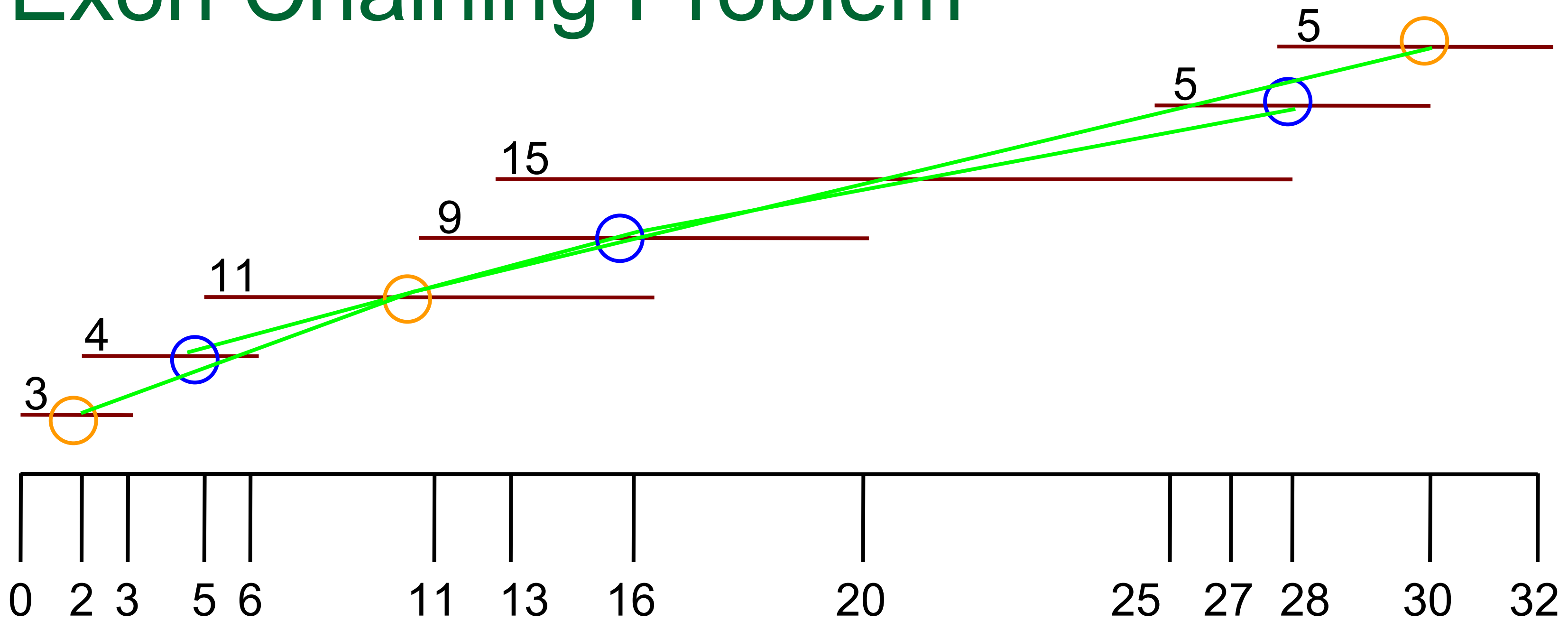
Chaining Local Alignments

- Find substrings that match a given gene sequence (candidate exons)
- Define a candidate exons as

  (l, r, w)

  (left, right, weight defined as score of local alignment)
- Look for a maximum chain of substrings
  - Chain: a set of non-overlapping nonadjacent intervals.

Exon Chaining Problem

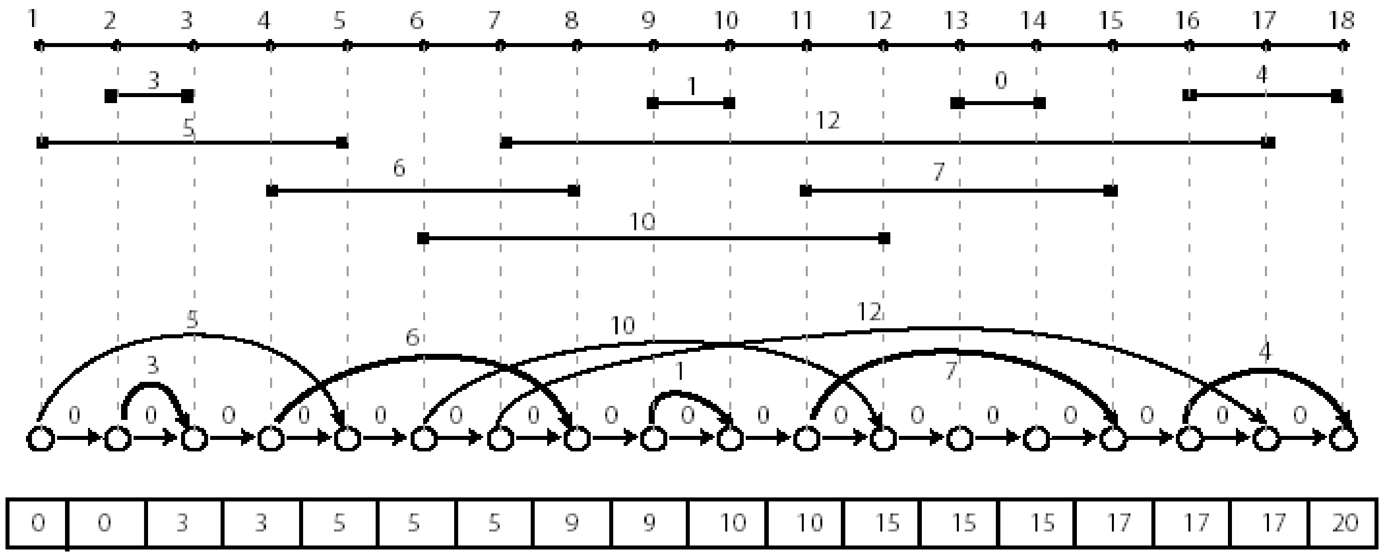


- Locate the beginning and end of each interval (2*n* points)
- Find the "best" path

Exon Chaining Problem: Formulation

- **Exon Chaining Problem:** Given a set of putative exons, find a maximum set of non-overlapping putative exons.
- **Input**: a set of weighted intervals (putative exons).
- **Output**: A maximum chain of intervals from this set.

Exon Chaining Problem: Graph Representation

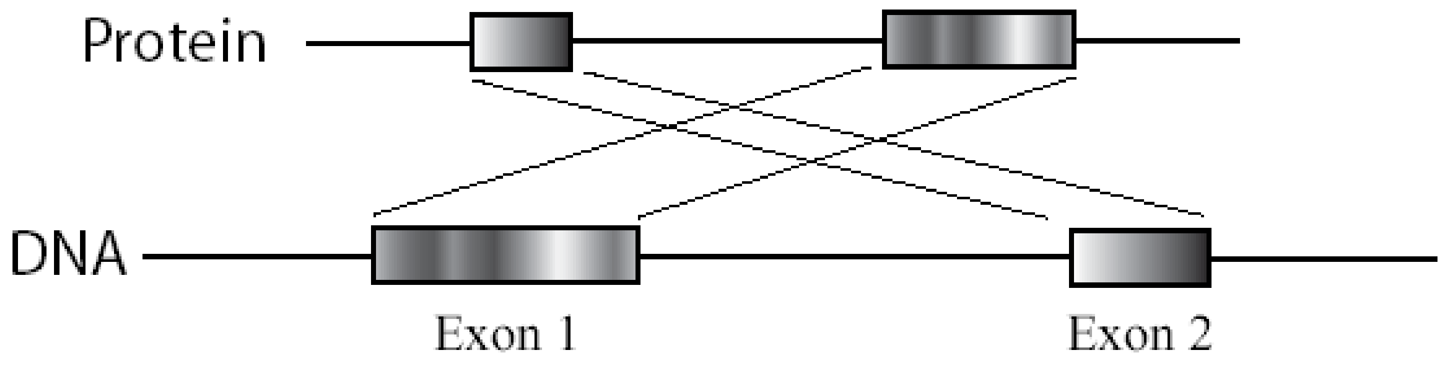


- This problem can be solved with dynamic programming in O(*n*) time.

Exon Chaining Algorithm

```
ExonChaining (G, n) //Graph, number of intervals
    for i ← to 2n
      $s_i \leftarrow 0$
    for i ← 1 to 2n
        if vertex $v_i$ in G corresponds to right end of the interval I
            j ← index of vertex for the left end of the interval I
            w ← weight of the interval I
            $s_j \leftarrow \max\{s_j + w, s_{i-1}\}$
        else
            $s_i \leftarrow s_{i-1}$
    return $s_{2n}$
```

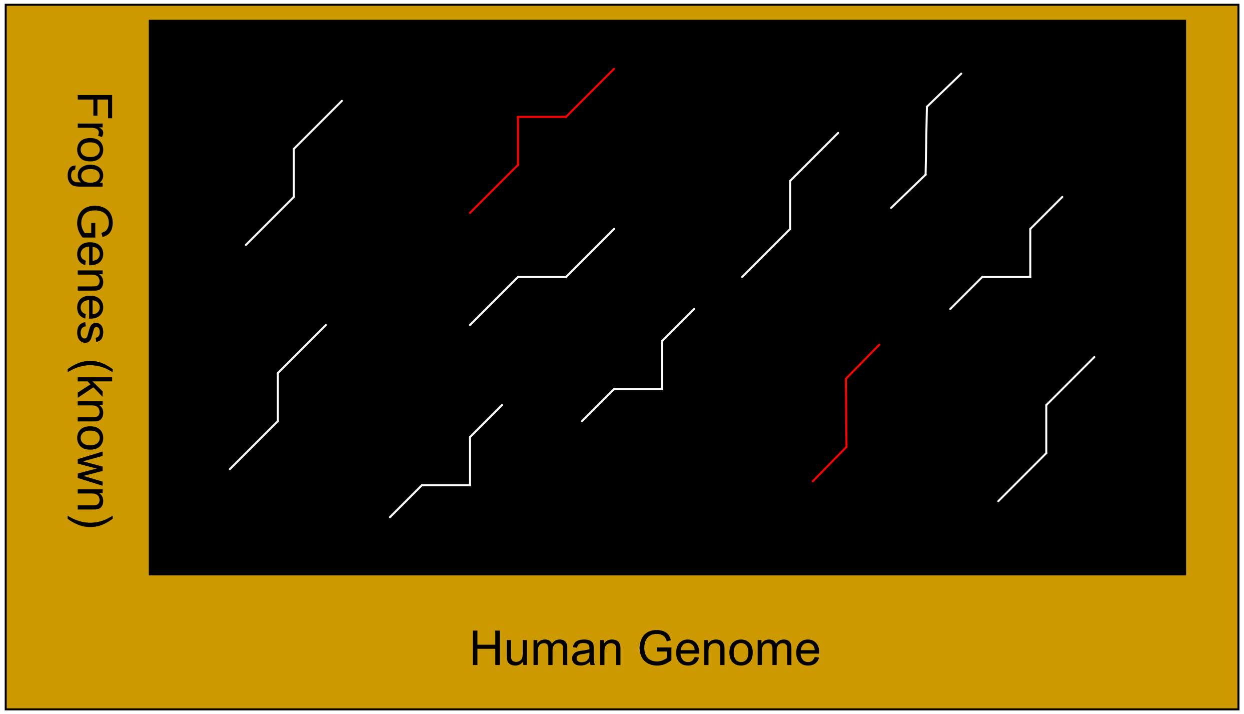
Exon Chaining: Deficiencies



- Poor definition of the putative exon endpoints.
- Optimal chain of intervals may not correspond to any valid alignment:
  - First interval may correspond to a suffix, whereas second interval may correspond to a prefix.
  - Combination of such intervals is not a valid alignment.

Infeasible Chains

- Red local similarities form two non -overlapping intervals but do not form a valid global alignment



Spliced Alignment

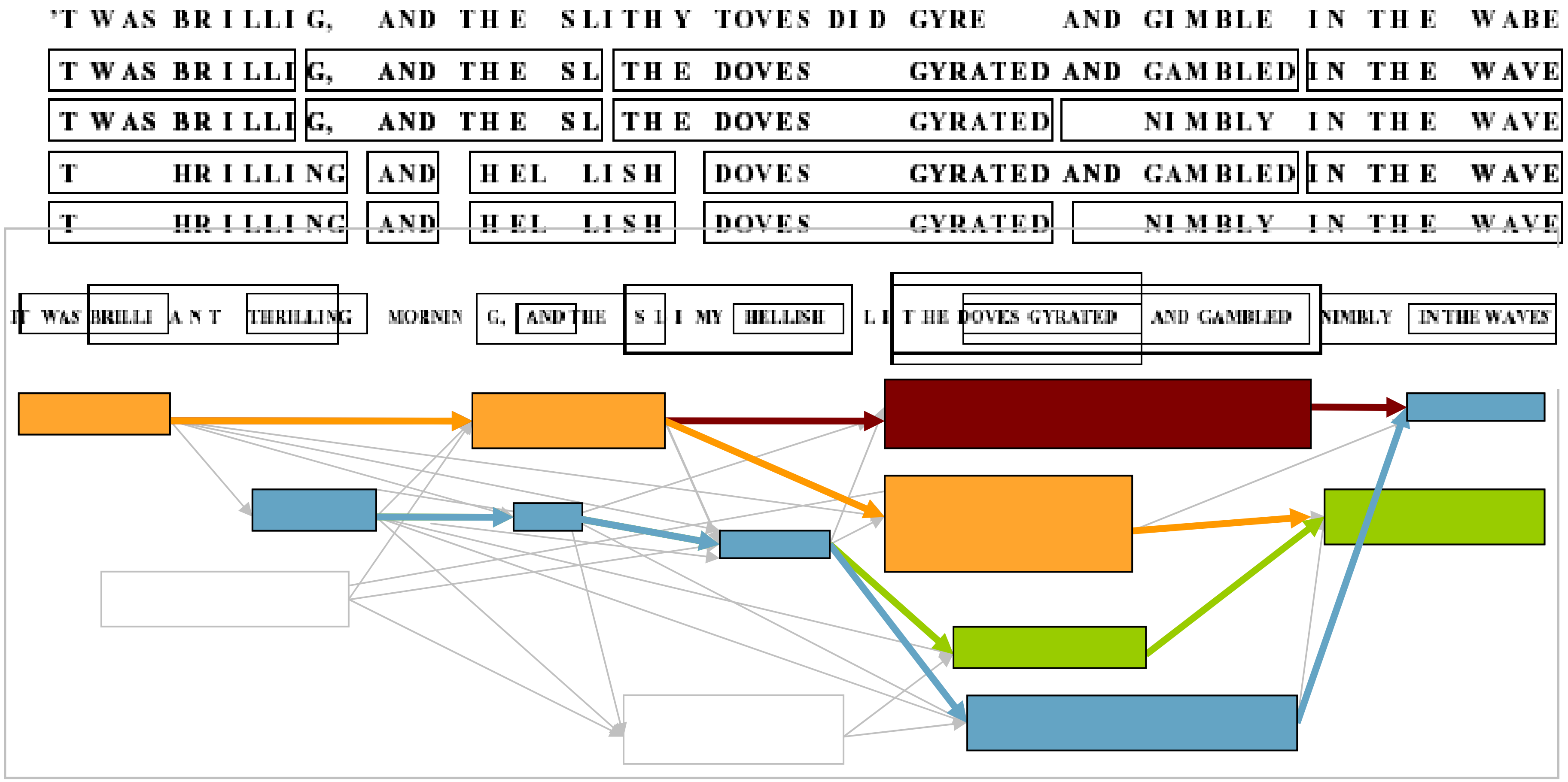
- Mikhail Gelfand and colleagues proposed a **spliced alignment** approach of using a protein within one genome to reconstruct the exon-intron structure of a (related) gene in another genome.
  - Begins by selecting either all putative exons between potential acceptor and donor sites or by finding all substrings similar to the target protein (as in the Exon Chaining Problem).
  - This set is further filtered in such way that attempts to retain all true exons, with some false ones.

Spliced Alignment Problem: Formulation

- **Goal**: Find a chain of blocks in a genomic sequence that best fits a target sequence
- **Input**: Genomic sequences *G*, target sequence *T*, and a set of candidate exons *B*.
- **Output**: A chain of exons Γ such that the global alignment score between Γ* and T is maximum among all chains of blocks from *B*.

Γ* – the concatenation of all exons from chain Γ

Lewis Carroll Example


'T WAS BRILLIG, AND THE SLITHY TOVES DID GYRE AND GIMBLE IN THE WABE
T WAS BRILLI G, AND THE SL THE DOVES GYRATED AND GAMBLED IN THE WAVE
T WAS BRILLI G, AND THE SL THE DOVES GYRATED NIMBLY IN THE WAVE
T HRILLING AND HEL LISH DOVES GYRATED AND GAMBLED IN THE WAVE
T HRILLING AND HEL LISH DOVES GYRATED NIMBLY IN THE WAVE
IT WAS BRILLI ANT THRILLING MORNIN G, AND THE S L I MY HELLISH L I T HE DOVES GYRATED AND GAMBLED NIMBLY IN THE WAVES

Spliced Alignment: Idea

- Compute the best alignment between *i*-prefix of genomic sequence *G* and *j*-prefix of target *T:*

  $S(i,j)$

- But what is "*i*-prefix" of *G*?
- There may be a few *i*-prefixes of *G* depending on which block *B* we are in.

Spliced Alignment: Idea

- Compute the best alignment between *i*-prefix of genomic sequence *G* and *j*-prefix of target *T:*

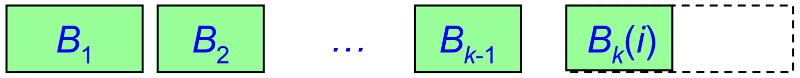
  S(i,j)

- But what is "*i*-prefix" of *G?*
- There may be a few *i*-prefixes of *G* depending on which block *B* we are in.
- Compute the best alignment between *i*-prefix of genomic sequence *G* and *j*-prefix of target *T* ***under the assumption*** that the alignment uses the block *B* at position *i*

  S(i,j,B)

The score of a prefix alignment

- Given a position i, let $\Gamma = (B_1, \ldots, B_k, \ldots, B_t)$ be a chain such that some block B_k contains i.
- We define



$$\Gamma^*(i) = B_1 * B_2 * \ldots * B_k(i)$$

as the concatenation of $B_1 \ldots B_{k-1}$ and the i-prefix of B_k.

- Then

$$S(i, j, k) = \max_{\text{all chains } \Gamma \text{ containing block } Bk} s(\Gamma^*(i), T(j)),$$

is the optimal score for aligning a chain of blocks up to position i in G to the j-prefix of T.

- The values of this matrix can be computed using dynamic programming.

Spliced Alignment Initialization

1. For j=0, $S(i, 0, B)$ corresponds to an aligment of blocks in front of B to gaps and $g_{first(B)} \ldots g_i$ to gaps

$$S(i,0,B) = \sum_{l=first(B)}^{i} indel$$

1. For j>0 and when there does not exist a block preceding B

$$S(i,j,B) = s(g_{first(B)} \ldots g_i, t_1 \ldots t_j)$$

- *first(B)* = index of the first base of B

Spliced Alignment Recurrence

- If i is not the starting vertex of block B:
  $S(i, j, B) =$
  $$\max \begin{cases} S(i-1, j, B) - \textit{indel penalty} \\ S(i, j-1, B) - \textit{indel penalty} \\ S(i-1, j-1, B) + \delta(g_i, t_j) \end{cases}$$

- If i is the starting vertex of block B:
  $S(i, j, B) =$
  $$\max \begin{cases} S(i, j-1, B) - \textit{indel penalty} \\ \max_{\text{all blocks } B' \text{ preceding block } B} S(end(B'), j, B') - \textit{indel penalty} \\ \max_{\text{all blocks } B' \text{ preceding block } B} S(end(B'), j-1, B') + \delta(g_i, t_j) \end{cases}$$

- After computing the three-dimensional table $S(i, j, B)$, the score of the optimal spliced alignment is:
  $$\max_{\text{all blocks } B} S(end(B), length(T), B)$$

Spliced Alignment: Complications

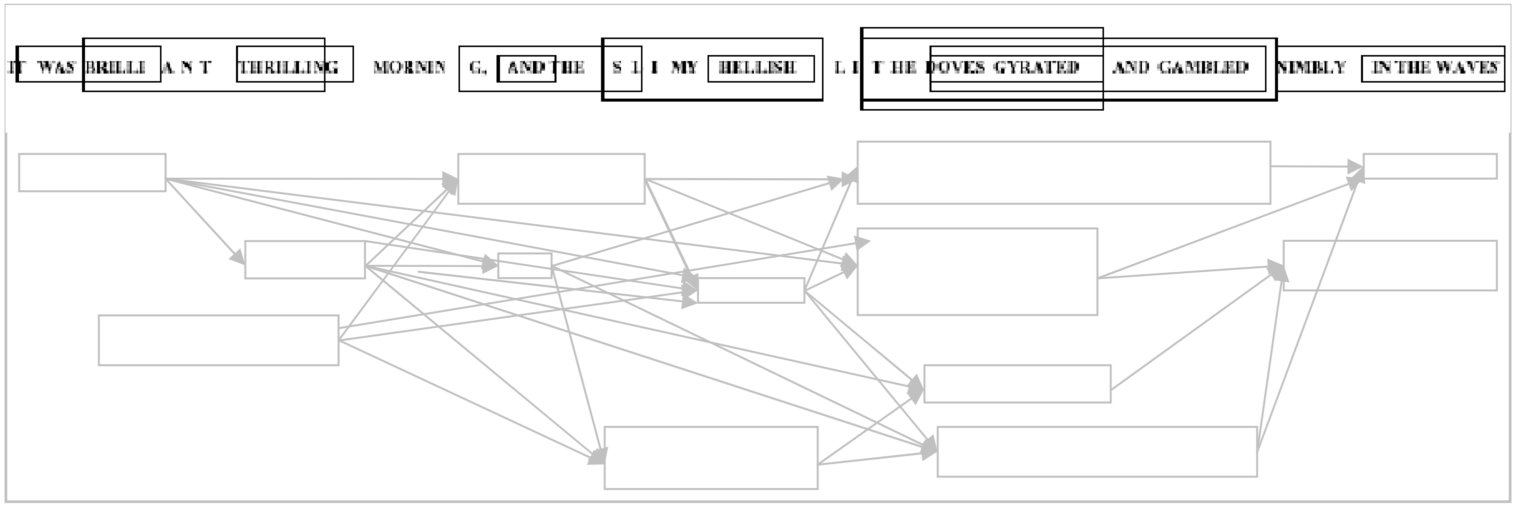
- Considering multiple *i*-prefixes leads to slow down. running time:

  $$O(mn^2\ |B|)$$

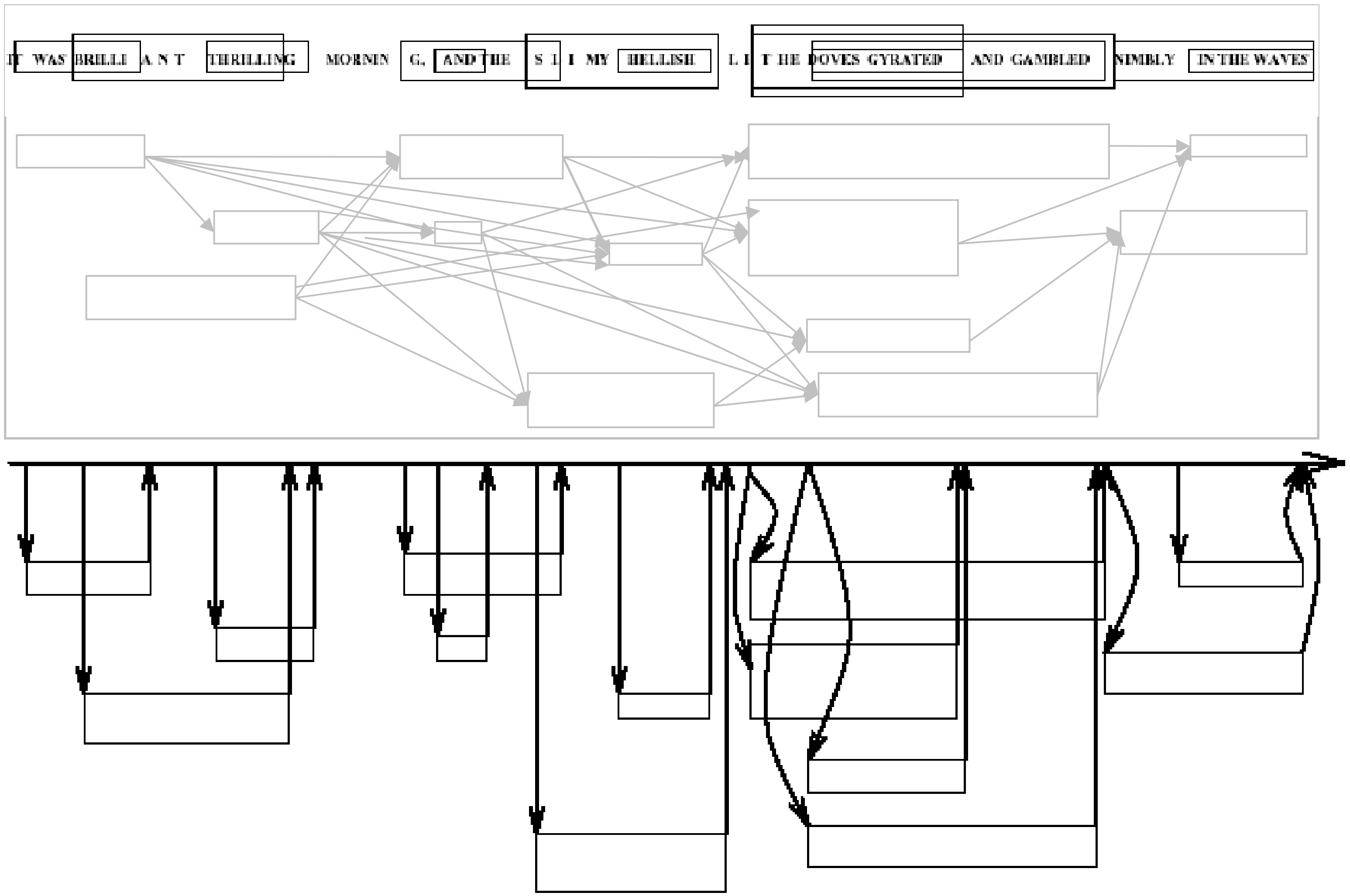
  where m is the target length, n is the genomic sequence length and $|B|$ is the number of blocks.

- A ***mosaic effect***: short exons are easily combined to fit any target protein

Spliced Alignment: Speedup

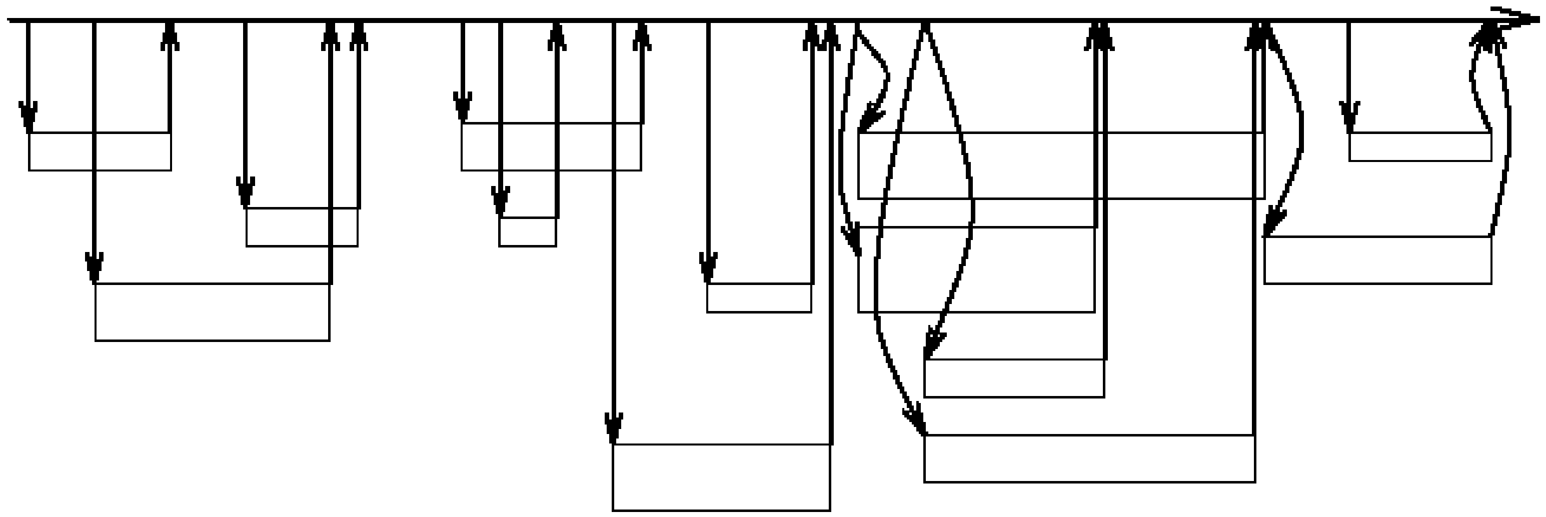

IT WAS BRILLI A N T THRILLING MORNIN G, AND THE S L I MY HELLISH L I T HE DOVES GYRATED AND GAMBLED NIMBLY IN THE WAVES

Spliced Alignment: Speedup


IT WAS BRILLI A N T THRILLING MORNIN G, AND THE S L I MY HELLISH L I T HE DOVES GYRATED AND GAMBLED NIMBLY IN THE WAVES

Spliced Alignment: Speedup

- $P(i,j)=\max_{\text{all blocks } B \text{ preceding position } i} S(end(B), j, B)$



Exon Chaining vs Spliced Alignment

- In Spliced Alignment, every path spells out string obtained by concatenation of labels of its edges. The weight of the path is defined as optimal alignment score between concatenated labels (blocks) and target sequence
  - Defines weight of entire path in graph, but not the weights for individual edges.
- Exon Chaining assumes the positions and weights of exons are pre-defined.

Gene Prediction: Aligning Genome vs. Genome

- Align entire human and mouse genomes.
- Predict genes in both sequences simultaneously as chains of aligned blocks (exons).
- This approach does not assume any annotation of either human or mouse genes.

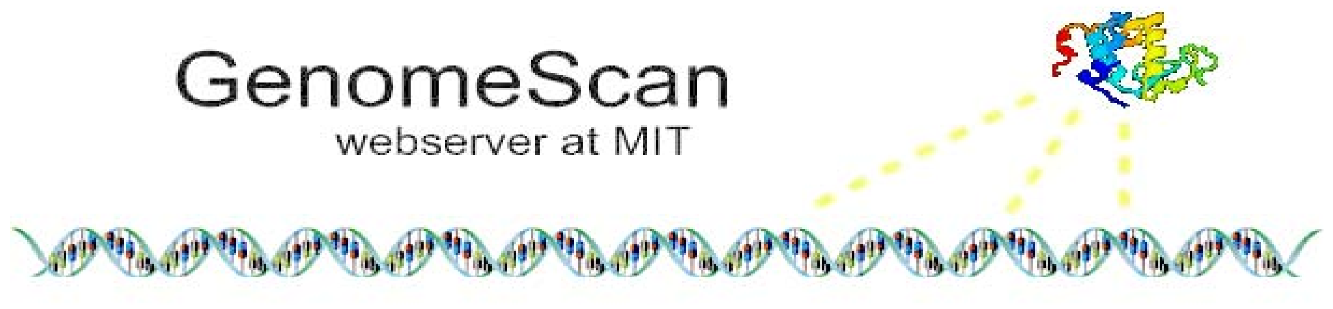
Gene Prediction Tools

- GENSCAN/Genome Scan
- TwinScan
- Glimmer
- GenMark

The GENSCAN Algorithm

- Algorithm is based on probabilistic model of gene structure similar to *Hidden Markov Models (HMMs).*
- GENSCAN uses a training set in order to estimate the *HMM parameters*, then the algorithm returns the exon structure using maximum likelihood approach standard to many HMM algorithms (*Viterbi* algorithm).
  - Biological input: Codon bias in coding regions, gene structure (start and stop codons, typical exon and intron length, presence of promoters, presence of genes on both strands, etc)
  - Covers cases where input sequence contains no gene, partial gene, complete gene, multiple genes.
- GENSCAN limitations:
  - Does not use similarity search to predict genes.
  - Does not address alternative splicing.
  - Could combine two exons from consecutive genes together

GenomeScan



- Incorporates similarity information into GENSCAN: predicts gene structure which corresponds to maximum probability conditional on similarity information
- Algorithm is a combination of two sources of information
  - Probabilistic models of exons-introns
  - Sequence similarity information

TwinScan

- Aligns two sequences and marks each base as gap (-), mismatch (:), match (|), resulting in a new alphabet of 12 letters: Σ = {A-, A:, A |, C-, C:, C |, G-, G:, G |, T-, T:, T|}.
- Run Viterbi algorithm using emissions $e_k(b)$ where $b \in \Sigma$, k.
- The emission probabilities are estimated from human/mouse gene pairs.
  - Ex. $e_I(x|) < e_E(x|)$ since matches are favored in exons, and

    $$e_I(x-) > e_E(x-)$$

    since gaps (as well as mismatches) are favored in introns.
  - Compensates for dominant occurrence of poly-A region in introns

Glimmer

- Gene Locator and Interpolated Markov ModelER
- Finds genes in bacterial DNA
- Uses interpolated Markov Models
- Made of 2 programs
  - **BuildIMM**
    - Takes sequences as input and outputs the Interpolated Markov Models (IMMs)
  - **Glimmer**
    - Takes IMMs and outputs all candidate genes
    - Automatically resolves overlapping genes by choosing one, hence limited
    - Marks “suspected to truly overlap” genes for closer inspection by user

GenMark

- Based on *non-stationary* Markov chain models
- Results displayed graphically with coding vs. noncoding probability dependent on position in nucleotide sequence