Gene finding

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Introduction

Gene finding is about detecting coding regions and infer gene structure

Gene finding is difficult

- DNA sequence signals have low information content (degenerated and highly unspecific)
- It is difficult to discriminate real signals
- Sequencing errors

Prokaryotes

- High gene density and simple gene structure
- Short genes have little information
- Overlapping genes

Eukaryotes

- Low gene density and complex gene structure
- Alternative splicing
- Pseudo-genes

Gene finding strategies

Homology method

- Gene structure can be deduced by homology
- Requires a not too distant homologous sequence

Ab initio method

- Requires two types of information
  - compositional information
  - signal information

Gene finding: Homology method

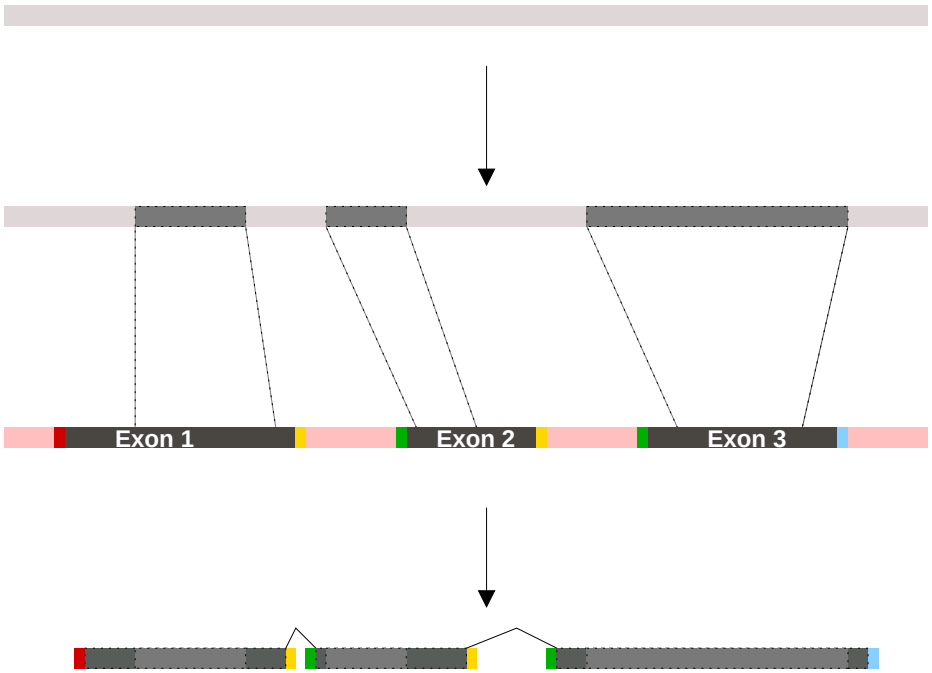
Homology method

Principles of the homology method.

- Coding regions evolve slower than non-coding regions, i.e. local sequence similarity can be used as a gene finder.
- Homologous sequences reflect a common evolutionary origin and possibly a common gene structure, i.e. gene structure can be solved by homology (mRNAs, ESTs, proteins, domains).
- Standard homology search methods can be used (BLAST, Smith-Waterman, ...).
- Include ”gene syntax” information (start/stop codons, ...).

Homology methods are also useful to confirm predictions inferred by other methods

Homology method: a simple view



Gene of unknown structure

Homology with a gene of known structure

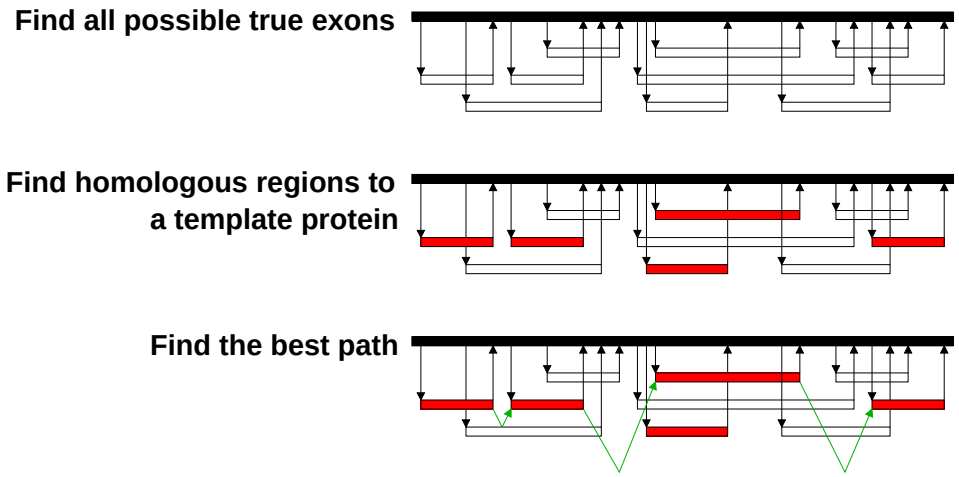
Find DNA signals

- ATG
- GT
- {TAA,TGA,TAG}
- AG

Procrustes

Procrustes is a software to predict gene structure from homology found in proteins (*Gelfand et al., 1996*)

- Principle of the algorithm
  - Find all possible blocks (exons) in the query sequence (based on the acceptor/donor sites)
  - Find optimal alignments between blocks and model sequences
  - Find the best alignment between concatenation of the blocks and the target sequence



Procrustes

Advantages of the homology method

- Successfully recognizes short exons and exons with unusual codon usage
- Assembles correctly complex genes (> 10 exons)
- Available on the web `http://www-hto.usc.edu/software/procrustes/qpn.html`

Problems of the homology method

- Genes without homologous in the databases are missed
- Requires close homologous to deduce gene structure
- Very sensitive to frame shift errors

Protocol to find gene structure using protein homology

- Do a BLASTX of your query sequence against a protein database (SWISS-PROT/TrEMBL)
- Retrieve sequences giving the best results
- Find gene structure using the retrieved sequences from the BLASTX search (Procrustes)
- BLAST the predicted protein against a protein database to verify the predicted gene structure

Genewise

Genewise uses HMMs to compare DNA sequences at the level of its conceptual translation, regardless of sequencing errors and introns.

Principle

- The gene model used in genewise is a HMM with 3 base states (match, insert, delete) with the addition of more transition between states to consider frame-shifts.
- Intron states have been added to the base model.
- Genewise directly compare HMM-profiles of proteins or domains to the gene structure HMM model.

Genewise can be used with the whole Pfam protein domain databases (find protein domain signatures in the DNA sequence).

Genewise is a powerful tool, but time consuming.

Genewise is part of the Wise2 package: http://www.sanger.ac.uk/Software/Wise2.

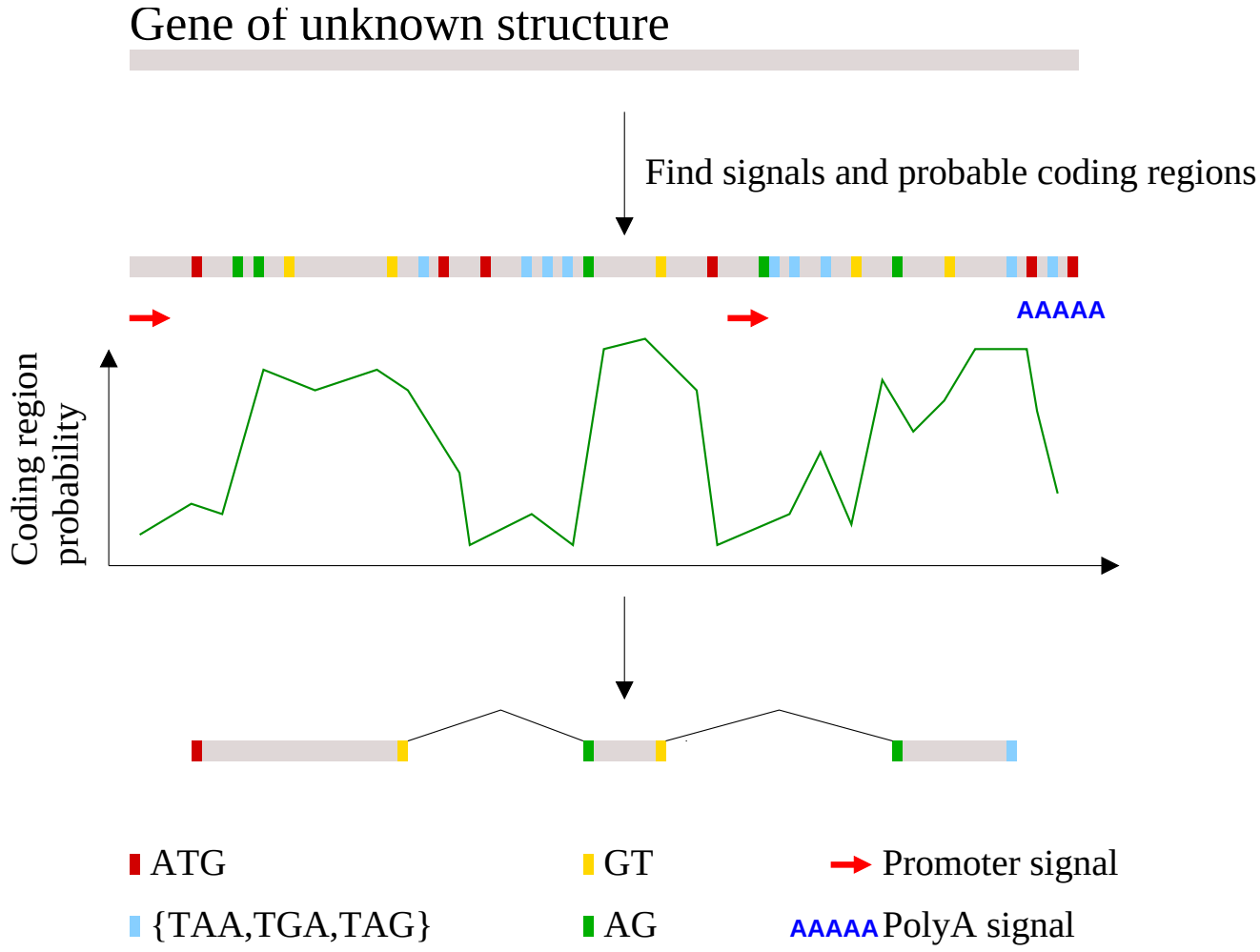
Gene finding: Ab initio method

Ab initio method

Principles of the ab initio methods

- Integration of signal detection and coding statistics
- Signal detection and coding statistics are deduced from a training set
- Probabilistic frameworks are used to infer a probable gene structure
- A solid scoring system can be used to evaluate the predictions

Ab initio method



Signal detection

Detect short DNA motifs (promoters, start/stop codons, splice sites,...).

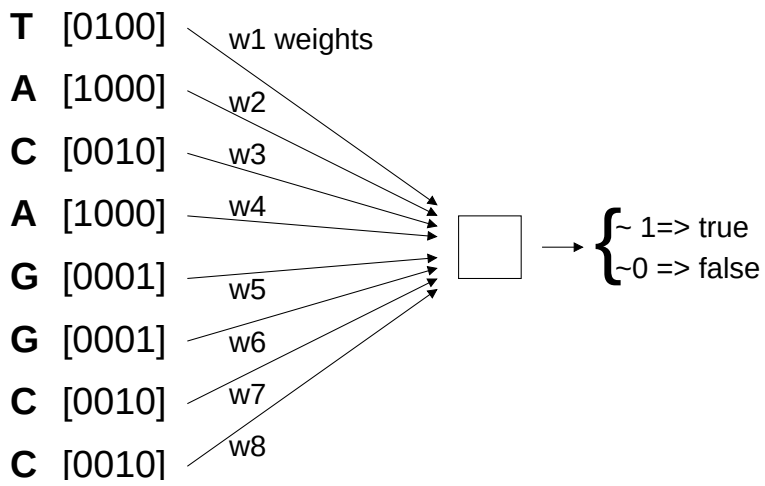
A number of methods are used for signal detection:

- Consensus string: based on most frequently observed residues at a given position.
- Pattern recognition: flexible consensus strings.
- Weight matrices: based on observed frequencies of residues at a given position. Uses standard alignment algorithms. This method returns a score.
- Weight array matrices: weight matrices based on dinucleotides frequencies. Takes into account the non-independence of adjacent positions in the sites.
- Maximal dependence decomposition (MDD): MDD generates a model which captures significant dependencies between non-adjacent as well as adjacent positions, starting from an aligned set of signals.

Signal detection

Methods for signal detection (continuation)

- Hidden Markov Models (HMM)
  - HMM uses a probabilistic framework to infer the probability that a sequence correspond to a real signal
- Neural Networks (NN)
  - NN are trained with positive and negatives example and ”discover” the features that distinguish the two sets.
    Example: NN for acceptor sites, the *perceptron*, *(Horton and Kanehisa, 1992)*



Signal detection

Signal detection problem

- DNA sequence signals have low information content
- Signals are highly unspecific and degenerated
- Difficult to distinguish between true and false positive

How improve signal detection

- Take context into consideration (ex. acceptor site must be flanked by an intron and an exon)
- Combine with coding statistics (compositional bias)

Coding statistics

Inter-genic regions, introns, exons, ... have different nucleotides contents

This compositional differences can be used to infer gene structure

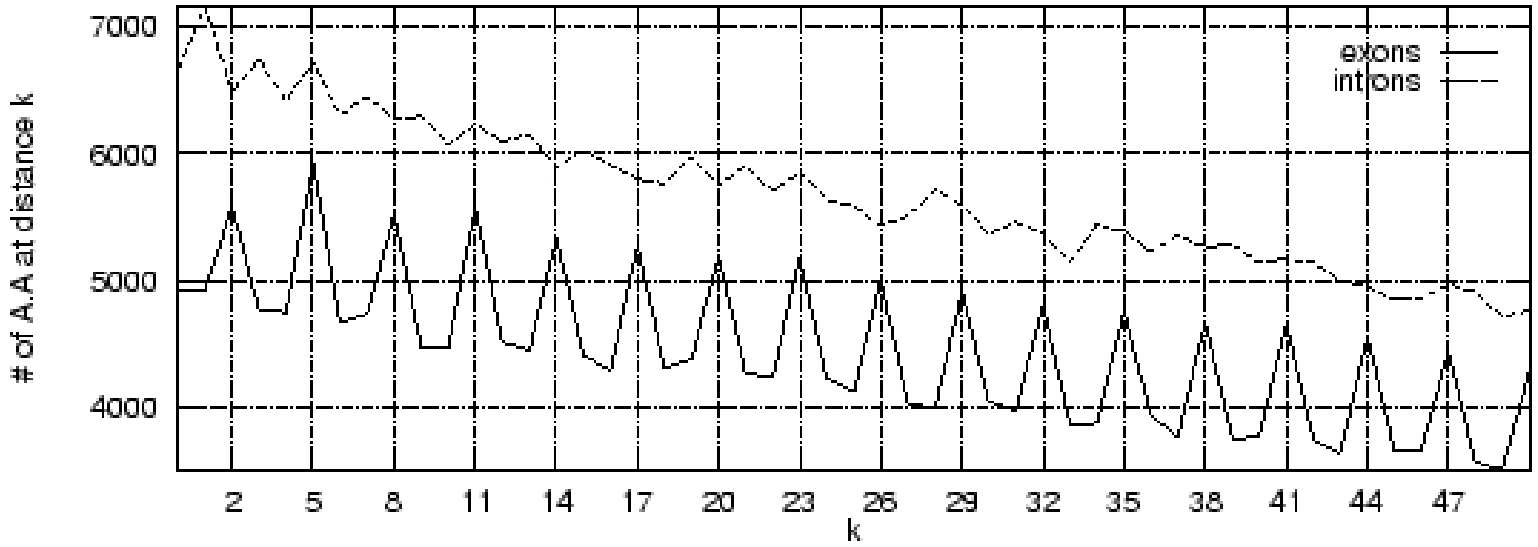
Examples of coding region finding methods:

- ORF length
  - Assuming an uniform random distribution, stop codons are present every 64/3 codons (≈ 21 codons) in average
  - In coding regions stop codon average decrease
  - Method sensitive to frame shift errors
  - Can't detect short coding regions
- Bias in nucleotide content in coding regions
  - Generally coding regions are G+C rich
  - There are exceptions. For example coding regions of *P. falciparum* are A+T rich

Coding statistics

Examples of coding region finding methods (continuation):

- Periodicity
  - Plot of the number of residues separating a pair of nucleotides is periodic in coding regions, but not in non-coding regions.



From *Guigó*, ”Genetic Databases”, Academic Press, 1999.

Codon frequencies

- Codon frequencies
  - Synonym codon usage is biased in a species dependent way
  - 3^{rd} codon position: 90% are A/T; 10% are G/C

- How to calculate codon frequencies
  Assume S = $a_1b_1c_1, a_2b_2c_2, \ldots, a_{n+1}b_{n+1}c_{n+1}$ is a coding sequence with unknown reading frame. Let f_{abc} denote the appearance frequency of codon abc in a coding sequence.
  The probabilities p_1, p_2, p_3 of observing the sequence of n codons in the 1^{st}, 2^{nd} and 3^{rd} frame respectively are:

$$p_1 = f_{a_1b_1c_1} \times f_{a_2b_2c_2} \times \ldots \times f_{a_nb_nc_n}$$
$$p_2 = f_{b_1c_1a_2} \times f_{b_2c_2a_3} \times \ldots \times f_{b_nc_na_{n+1}}$$
$$p_3 = f_{c_1a_2b_2} \times f_{c_2a_3b_3} \times \ldots \times f_{c_na_{n+1}b_{n+1}}$$

  The probability P_i of the ith reading frame for being the coding region is:

$$P_i = \frac{p_i}{p_1+p_2+p_3}$$

  where $i \in \{1, 2, 3\}$.

Codon frequencies

In practice we use these computations in a search algorithm as follows:

- Select a window of size n (for example $n = 30$)
- Slide the window along the sequence and calculate P_i for each start position of the window

A variation of the codon frequency method is to use 6-tuple frequencies instead of 3-tuple (codon) frequencies. This method was found to be the best single property to predict whether a window of vertebrate genomic sequence was coding or non-coding *(Claverie and Bougueleret, 1986)*.

The usage of hexamers frequencies has been integrated in a number of gene predictors.

Integrating signal information and compositional information for gene structure prediction

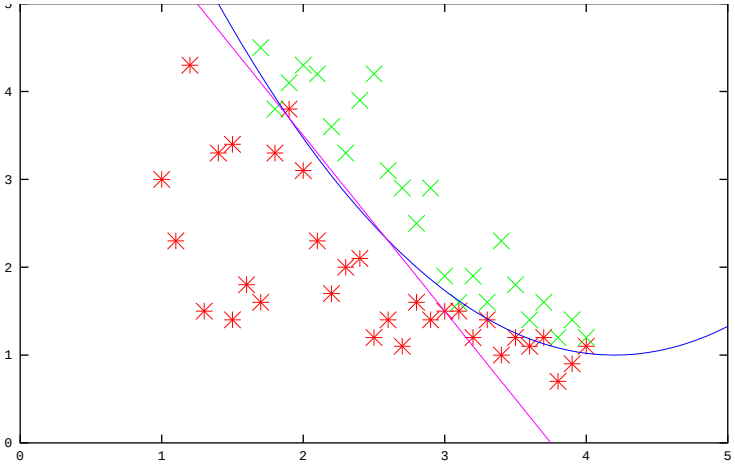
A number of methods exists for gene structure prediction which integrate different techniques to detect signals (splicing sites, promoters, etc.) and coding statistics.

The following slides will present a non-exhaustive list of these methods.

Integrating signal information and compositional information for gene structure prediction

Linear and quadratic discrimination analysis

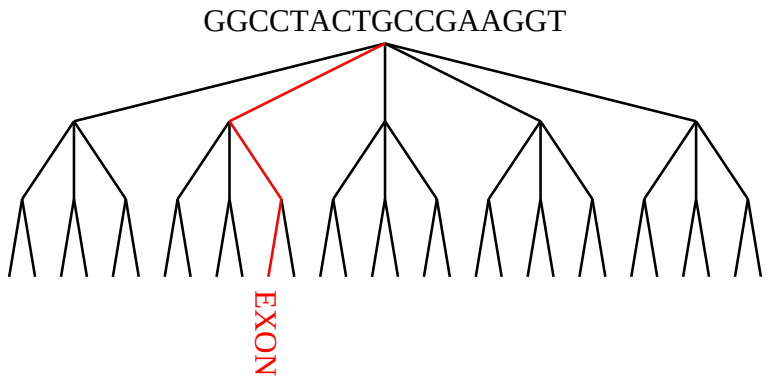
- Linear discrimination analysis is a standard technique in multivariate analysis.
- Linear discrimination analysis is used to linearly combine several measures in order to perform the best discrimination between coding and non-coding sequences.
- Quadratic discriminant analysis. Similar to linear discrimination analysis, but uses a quadratic discriminant function
- Dynamic programming is used in to combine the inferred exons



Integrating signal information and compositional information for gene structure prediction

Decision tree

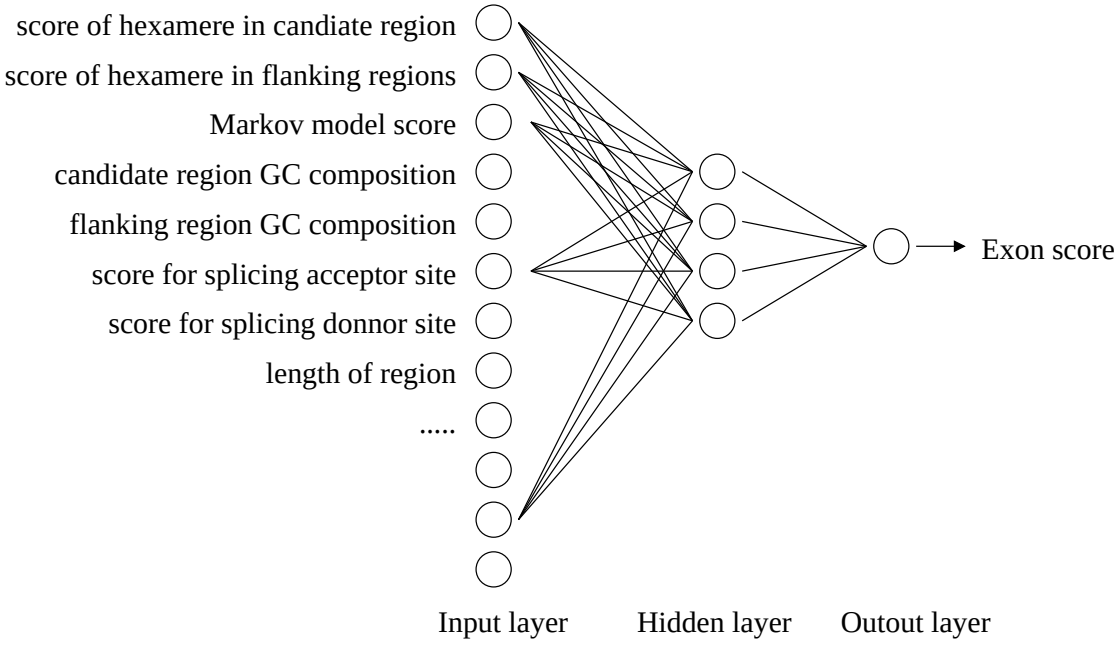
- Internal nodes of a decision tree are property values tested for each subsequence passed to the tree
- Properties can be various coding measures (e.g. hexamers frequencies) or signal strengths
- Bottom nodes (leaves) of the tree contains class labels to be associated with the sub-sequences
- Dynamic programming is used to deduce the complete gene structure



Integrating signal information and compositional information for gene structure prediction

Neural network

- The neural network is trained with a set of true positives and true negatives examples
- For each training example, the neurons are tuned to return the right answer
- Dynamic programming is used to deduce the complete gene structure



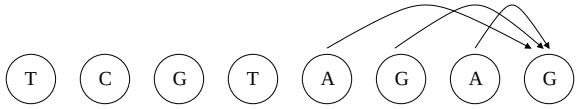
(Uberbacher et al., 1996)

Integrating signal information and compositional information for gene structure prediction

Markov Model (MM)

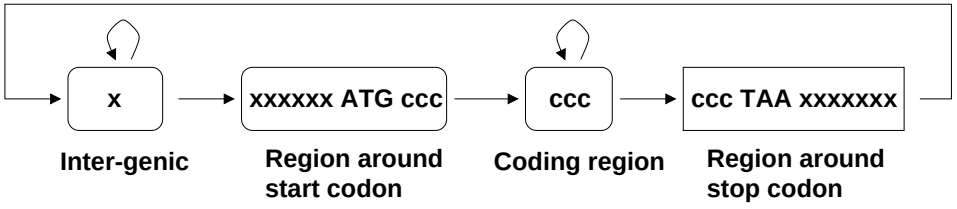
- Biological sequences can be modeled as the output of a stochastic process in which the probability for a given nucleotide to occur at position p depends on the k previous positions. This representation is called k-order Markov Model.

$$P(x_i|x_1, x_2, \ldots, x_{i-1}) = P(x_i|x_{i-k}, x_{i-(k-1)}, \ldots, x_{i-1})$$



Hidden Markov Model (HMM)

- In a HMM the biological sequences are modeled as the output of a stochastic process that progresses through a series of discrete states. Each state model correspond to a Markov Model.



(Krog, 1998)

Integrating signal information and compositional information for gene structure prediction

Generalized Hidden Markov Model (GHMM)

- GHMMs are HMMs where states are arbitrary sub-models (e.g., neural networks, position weight matrices, etc.).
- The duration of a particular state depends on some probability distributions.

Principle of Markov Models

- Given a DNA string S, find the most probable path M in the model that generates S. This will be the most probable gene structure.

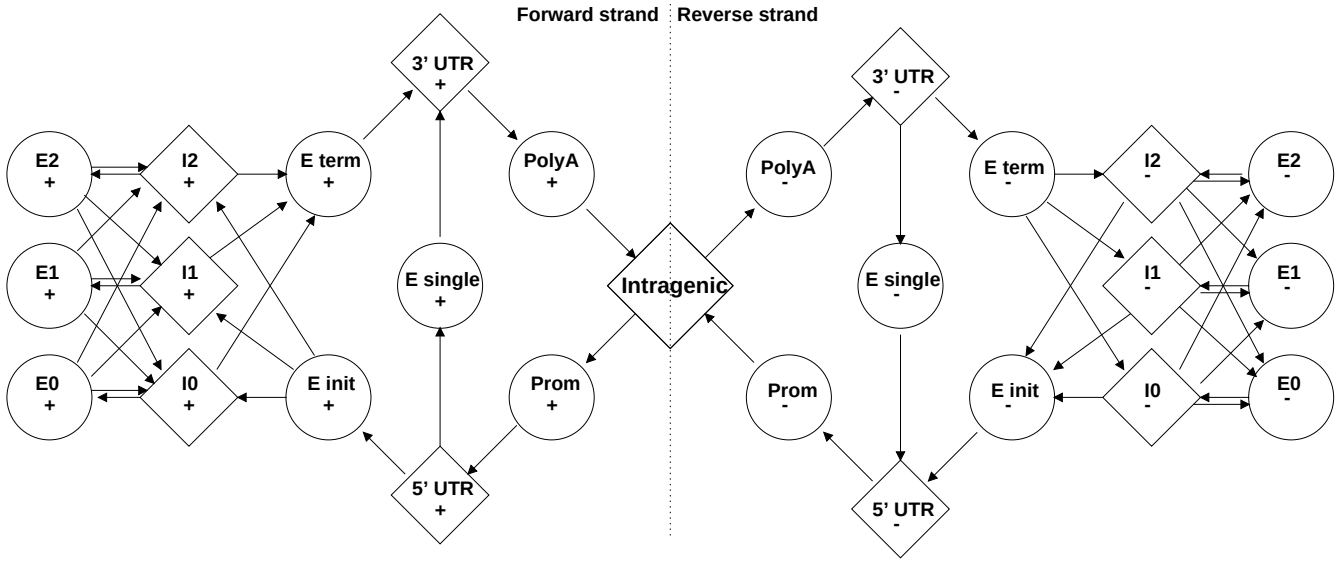
Markov derived models have many desirable properties

- Modeling: theoretically well-founded models.
- Efficient: $O(|M| \cdot |S|)$ where $|M|$ is the number of states in the model and $|S|$ is the length of the string.
- Scoring: theoretically well-founded scoring system.

Integrating signal information and compositional information for gene structure prediction

Example of the GHMM in GENSCAN *(Burge and Karlin, 1997)*

- Each state can correspond to a position weight matrix, a neural network, ...
- Each state has an associated length distribution (time) → there are no transitions from the state to the state itself.
- The underlying (hidden) model of GENSCAN:



Description of some gene predictors available on the web

GRAIL

GRAIL 1

- Neural network recognizing coding potential within a fixed-size window (100 bases)
- Evaluates coding potential without looking for additional features ($e.g.$, splice junctions, start/stop codons)
- Exon quality evaluated by a score

GRAIL 1a

- Look at regions immediately adjacent to regions with coding potential
- Determine the ”best” boundaries for the coding region
- Performs better than GRAIL 1 in finding true exons and eliminating false positives
- Returns a score

GRAIL

GRAIL 2

- Uses variable window length
- Incorporates genomic context information
  - Splice junctions
  - Start and stop codons
  - PolyA signals
- Requires regions next to an exon
- Not appropriate for sequences without genomic context
- Much better at estimating the true extent of an exon as compared to GRAIL 1
- Returns scores
- WEB server
  - *http://compbio.ornl.gov*
  - Accept multiple sequence
  - Length 100 bases to 100 kilobases
  - Available for Human, Mouse, Drosophila, Arabidopsis, and E. coli

GRAIL

GRAIL 2 output

- [grail2exons -> Exons]

```
      St Fr Start     End ORFstart ORFend     Score      Quality
   1-  f 1    479     666     452     670    52.000         good
   2-  f 0   5176    5290    5176    5370    82.000    excellent
   3-  f 2   5395    5562    5364    5618    99.000    excellent
   4-  f 0   7063    7113    7063    7113    53.000         good
   5-  f 0  11827   11899   11590   11925    74.000         good
   6-  f 0  12188   12424   12163   12633    88.000    excellent
   7-  f 0  14288   14623   14194   14640    94.000    excellent
   8-  f 0  17003   17203   16957   17235   100.000    excellent
   9-  f 0  17751   17859   17659   17988    50.000         good
  10-  f 1  18212   18264   18071   18268    61.000         good

[grail2exons -> Exon Translations]

  11- MLRGTDASNNSEVFKKAKIMFLEVRKSLTCGQGPTGSSCNGAGQRESGHA
AFGIKHTQSVDR

  12- AQIPNQQELKETTMCRAISLRRLLLLLLQLCKFSDLGT

  13- AQLLAVTQGKTLVLGKEGESAELPCESSQKKITVFTWKFSDQRKILGQHG
KGVLIR
```

FGENES

Linear discriminant analysis

- Combine several measures of pattern recognition using a linear discriminant analysis
  - Donor and acceptor splice sites
  - Putative coding regions
  - 5' and 3' intronic regions of the putative exon
- Pass the previous results to a dynamic programming algorithm to find a coherent gene model

Each predicted exon has an associated score, but not directly useful (most of the scores < 10, while probable good prediction scores > 10)

WEB server

- http://genomic.sanger.ac.uk/gf/gf.shtml
- Can combine homology method with ab initio results
- Available organisms
  - Human, Drosophila, Worm, Yeast, Plants

FGENES

FGENES output (CDSf = first exon; CDSi = internal exon; CDSl = last exon; CDSo = only one exon; PolA = PolyA signal)

- Length of sequence: 20000 GC content: 0.48 Zone: 2
  Number of predicted genes: 2 In +chain: 2 In -chain: 0
  Number of predicted exons: 12 In +chain: 12 In -chain: 0
  Predicted genes and exons in var: 2 Max var= 15 GENE WEIGHT: 27.3

G	Str	Feature	Start		End	Weight	ORF-start		ORF-end
1	+	1 CDSf	990	-	1032	1.84	990	-	1031
1	+	2 CDSl	1576	-	1835	0.89	1578	-	1832
1	+	PolA	3106			4.64			
2	+	1 CDSf	5215	-	5266	5.25	5215	-	5265
2	+	2 CDSi	5395	-	5562	3.08	5397	-	5561
2	+	3 CDSi	11464	-	11490	0.76	11466	-	11489
2	+	4 CDSi	11738	-	11899	3.28	11740	-	11898
2	+	5 CDSi	12188	-	12424	2.48	12190	-	12423
2	+	6 CDSi	14288	-	14623	3.26	14290	-	14622
2	+	7 CDSi	17003	-	17203	2.79	17005	-	17202
2	+	8 CDSi	17741	-	17859	1.62	17741	-	17857
2	+	9 CDSi	18197	-	18264	2.53	18196	-	18264
2	+	10 CDSl	18324	-	18630	0.87	18325	-	18627

```
Predicted proteins:
>FGENES-M 1.5 >MySeq            1 Multiexon gene     990 -    1835     100 a Ch+
MSSAFSDPFKEQNPVISLITRTNLNSSSLPVRIYCQPPNMFLYIAPCAVLVLSTSSTPRR
TENGPLRMALNSRFPASFYLLCRDYQYTPPQLGPLHGRCS
>FGENES-M 1.5 >MySeq            2 Multiexon gene    5215 -   18630     558 a Ch+
MCRAISLRRLLLLLLQLSQLLAVTQGKTLVLGKEGESAELPCESSQKKITVFTWKFSDQR
```

MZEF

- Designed to predict only internal coding exons
- To discriminate between coding and non-coding regions, MZEF uses ”quadratic discriminant analysis” of different measures
  - Exon length
  - Intron-exon transition/Exon-intron transition
  - Branch-site scores
  - 5’ and 3’ splice sites scores
  - Exon score
  - Strand score
- Possible to set a prior probability depending on the gene density and G+C content
- Scores over-estimate of the accuracy of the prediction
- WEB server: http://www.cshl.org/genefinder
  - Length up to 200 kilobases
  - Available organisms: Human, Mouse, Arabidopsis, Fission yeast

MZEF

MZEF output

- Internal coding exons predicted by MZEF
  Sequence_length: 19920 G+C_content: 0.475

Coordinates	P	Fr1	Fr2	Fr3	Orf	3ss	Cds	5ss
5315 - 5482	0.580	0.623	0.528	0.585	122	0.506	0.608	0.552
6475 - 6582	0.752	0.482	0.563	0.558	221	0.505	0.567	0.598
11658 - 11819	0.822	0.476	0.569	0.497	211	0.554	0.560	0.651
14208 - 14543	0.903	0.593	0.619	0.469	212	0.497	0.603	0.575

- Description of the symbols
  - P: Posterior probability (between .5 to 1.)
  - Fri: Frame preference score for the ith frame of the genomic sequence
  - Orf: ORF indicator,”011” (or ”211”) means 2nd and 3rd frames are open
  - 3ss: Acceptor score
  - Cds: Coding preference score
  - 5ss: Donor score

HMMgene

Designed to predict complete gene structure

Uses HMM with a criterion called ”Conditional Maximum Likelihood” which maximize the probability of correct predictions

Can return sub-optimal prediction to help identifying alternative spicing

Regions of the sequence can be locked as coding and non-coding by the user

Probability score for each predicted exon

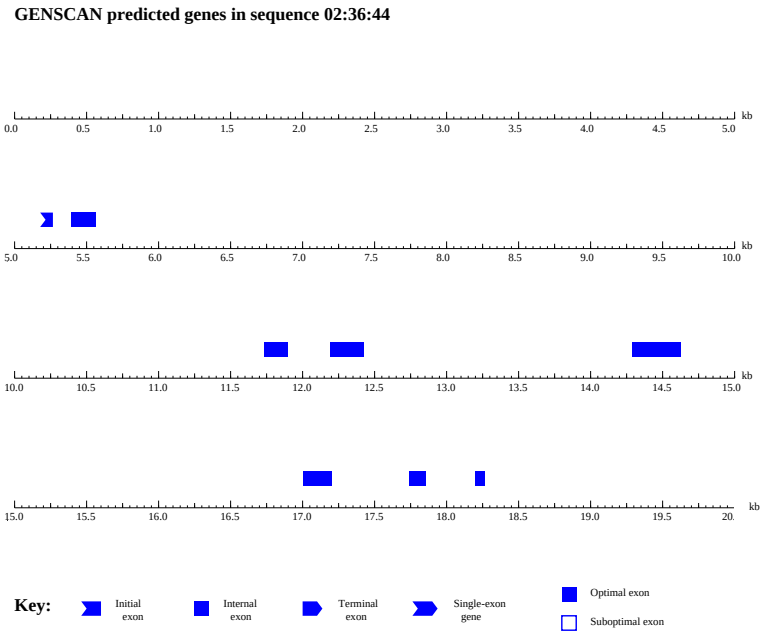
WEB server

- http://genome.cbs.dtu.dk/services/HMMgene
- Available organisms:
  - Human and worm

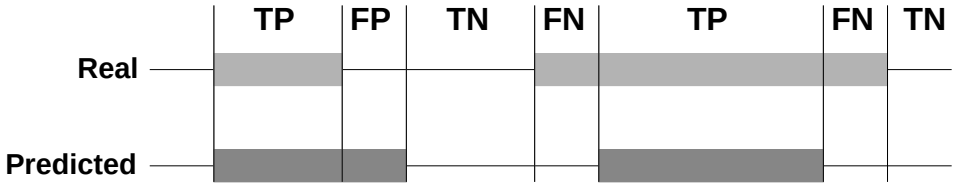
HMMgene

HMMgene output

- ```
  # SEQ: Sequence 20000 (-) A:5406 C:4748 G:4754 T:5092
  Sequence  HMMgene1.1a  firstex  17618  17828  0.578  -  1  bestparse:cds_1
  Sequence  HMMgene1.1a  exon_1   17049  17101  0.560  -  0  bestparse:cds_1
  Sequence  HMMgene1.1a  exon_2   14517  14607  0.659  -  1  bestparse:cds_1
  Sequence  HMMgene1.1a  exon_3   13918  13973  0.718  -  0  bestparse:cds_1
  Sequence  HMMgene1.1a  exon_4   12441  12508  0.751  -  2  bestparse:cds_1
  Sequence  HMMgene1.1a  lastex   7045   7222   0.893  -  0  bestparse:cds_1
  Sequence  HMMgene1.1a  CDS      7045   17828  0.180  -  .  bestparse:cds_1
  Sequence  HMMgene1.1a  DON      19837  19838  0.001  -  1
  Sequence  HMMgene1.1a  START    19732  19734  0.024  -  .
  Sequence  HMMgene1.1a  ACC      19712  19713  0.001  -  0
  Sequence  HMMgene1.1a  DON      19688  19689  0.006  -  1
  Sequence  HMMgene1.1a  DON      19686  19687  0.004  -  0
  ...
                                 ------------  -----  ----
                                 position      prob   strand and frame
  ```

- Symbols: firstex = first exon; exon_n = internal exon; lastex = last exon; singleex = single exon gene; CDS = coding region
  ```

GENSCAN

Designed to predict complete gene structure

Uses generalized hidden Markov models (GHMM): structure of genomic sequence is modeled by explicit state duration HMM

Signals are modeled by weight matrices, weight arrays, and maximal dependence decomposition

Probability score for each predicted exon

WEB server

- http://genes.mit.edu/GENSCAN.html
- Sequence length up to 200 kilobases
- Graphical output available
- Available organisms
  - Vertebrate, Arabidopsis, Maize

GENSCAN

GENSCAN output

- Gn.Ex Type S .Begin ...End .Len Fr Ph I/Ac Do/T CodRg P.... Tscr..

Gn.Ex	Type	S	.Begin	...End	.Len	Fr	Ph	I/Ac	Do/T	CodRg	P....	Tscr..
1.00	Prom	+	1653	1692	40							-1.16
1.01	Init	+	5215	5266	52	0	1	83	75	151	0.925	12.64
1.02	Intr	+	5395	5562	168	2	0	89	75	163	0.895	15.02
1.03	Intr	+	11738	11899	162	0	0	74	113	101	0.990	11.15
1.04	Intr	+	12188	12424	237	0	0	71	86	197	0.662	15.39
1.05	Intr	+	14288	14623	336	0	0	82	98	263	0.986	22.19
1.06	Intr	+	17003	17203	201	0	0	116	86	102	0.976	12.06
1.07	Intr	+	17741	17859	119	0	2	78	109	51	0.984	6.38
1.08	Intr	+	18197	18264	68	1	2	103	72	81	0.541	5.70

```
>02:36:44|GENSCAN_predicted_peptide_1|448_aa
MCRAISLRRLLLLLLQLSQLLAVTQGKTLVLGKEGESAELPCESSQKKITVFTWKFSDQR
KILGQHGKGVLIRGGSPSQFDRFDSKKGAWEKGSFPLIINKLKMEDSQTYICELENRKEE
...
Gn.Ex : gene number, exon number (for reference)
Type  : Init = Initial exon (ATG to 5' splice site)
        Intr = Internal exon (3' splice site to 5' splice site)
        Term = Terminal exon (3' splice site to stop codon)
        Sngl = Single-exon gene (ATG to stop)
        Prom = Promoter (TATA box / initation site)
        PlyA = poly-A signal (consensus: AATAAA)
S     : DNA strand (+ = input strand; - = opposite strand)
Begin : beginning of exon or signal (numbered on input strand)
End   : end point of exon or signal (numbered on input strand)
Len   : length of exon or signal (bp)
Fr    : reading frame (a forward strand codon ending at x has frame x mod 3)
```

```
Ph    : net phase of exon (exon length modulo 3)
I/Ac  : initiation signal or 3’ splice site score (tenth bit units)
Do/T  : 5’ splice site or termination signal score (tenth bit units)
CodRg : coding region score (tenth bit units)
P     : probability of exon (sum over all parses containing exon)
Tscr  : exon score (depends on length, I/Ac, Do/T and CodRg scores)
```

- Graphical output



GeneMark.hmm

Initially developed for bacterial gene finding

Recently modified to predict gene structure in eukaryotes

Uses explicit state duration HMM

Optimal gene candidates, selected by HMM and dynamic programming, are further processed by a ribosomal binding site recognition algorithm

No scores!

WEB server

- http://opal.biology.gatech.edu/GeneMark
- Access to both the original GeneMark and GeneMark.hmm

Geneid

One of the oldest gene structure prediction programs, recently updated to a new and faster version.

Uses a hierarchical search structure (signal $\longrightarrow$ exon $\longrightarrow$ gene):

- 1^{st}: finds signals (splice sites, start and stop codons);
- 2^{nd}: from the found signals start to score regions for exon-defining signals and protein-coding potential;
- 3^{rd}: a dynamic programming algorithm is used to search the space of predicted exons to assemble the gene structure.

Very fast and scale linearly with the length of the sequence (both in time and memory) $\Rightarrow$ adapted to analyze large sequences.

Trained with Drosophila and Human.

Available at http://www1.imim.es/geneid.html ... also sources!

Geneid

Geneid output

- ```
  ## date Mon Aug 26 19:14:40 2002
  ## source-version: geneid v 1.1 -- geneid@imim.es
  ## Sequence emb|U47924|HSU47924 - Length = 21000 bps
  # Optimal Gene Structure. 2 genes. Score = 60.238383
  # Gene 1 (Forward). 4 exons. 266 aa. Score = 19.588764
  Internal      419      67211.08+ 1 1 5.18 3.5027.17 0.00AA   1: 86 gene_1
  Internal     1325     1455 2.54+ 2 0 2.87 0.6013.64 0.00AA  86:129 gene_1
  Internal     3900     4036-0.01+ 0 2-0.38 2.5110.53 0.00AA 130:175 gene_1
  Terminal     6740     7013 5.99+ 1 0 0.87 0.0026.16 0.00AA 175:266 gene_1
  >emb|U47924|HSU47924|geneid_v1.1_predicted_protein_1|266_AA
  gDLTDIYLLRSYIHLRYVDISENHLTDLSPLNYLTHLLWLKADGNRLRSAQMNELPYLQI
  ASFAYNQITDTEGISHPRLETLNLKGNSIHMVTGLDPEKLISLHTVELRGNQLESTLGIN
  LPKLKNLYLAQNMLKKVEGLEDLSNLTTLHLRDNQIDTLSGFSREMKSLQYLNLRGNMVA
  NLGELAKLRDLPKLRALVLLDNPCTDETSYRQEALVQMPYLERLDKEFYEEEERAEADVI
  RQRLKEEKEQEPEPQRDLEPEQSLI*
  # Gene 2 (Forward). 7 exons. 395 aa. Score = 40.649619
     First     9843     9927 1.75+ 0 1 1.80 2.6910.15 0.00AA   1: 29 gene_2
  Internal    10427    10522 3.49+ 2 1 6.38 4.53 4.86 0.00AA  29: 61 gene_2
  Internal    11591    11724 2.44+ 2 0 1.72 3.0011.50 0.00AA  61:105 gene_2
  Internal    11950    1217214.65+ 0 1 4.72 4.5335.26 0.00AA 106:180 gene_2
  Internal    13576    13866 8.03+ 2 1 3.71 3.0522.43 0.00AA 180:277 gene_2
  Internal    15590    15791 8.94+ 2 2 3.73 1.6926.71 0.00AA 277:344 gene_2
  Internal    16256    16411 1.35+ 1 2 5.90 3.92 1.13 0.00AA 344:395 gene_2
  ```
  ```

Evaluating performances



Measures *(Burset and Guigo, 1996; Snyder and Stormo, 1997)*

- **Sensitivity** S_n is the proportion of coding nucleotides that are correctly predicted as coding:

$$S_n = \frac{TP}{TP+FN}$$

- **Specificity** S_p is the proportion of nucleotides predicted as coding that are actually coding:

$$S_p = \frac{TP}{TP+FP}$$

Evaluating performances

- **Correlation coefficient** CC is a single measure that captures both specificity and sensitivity:

$$CC = \frac{(TP \star TN) - (FN \star FP)}{\sqrt{(TP+FN) \star (TN+FP) \star (TP+FP) \star (TN+FN)}}$$

- **Approximate correlation** AC is similar to CC, but defined under any circumstances:

$$AC = (ACP - 0.5) \star 2$$

where

$$ACP = \frac{1}{4}\left(\frac{TP}{TP+FN} + \frac{TP}{TP+FP} + \frac{TN}{TN+FP} + \frac{TN}{TN+FN}\right)$$

Accuracy of the different methods

Evaluation of the different programs *(Rogic et al., 2001)*

Programs	No. of sequences	S_n	S_p	AC	CC
FGENES	195	0.86	0.88	0.84 ± 0.19	0.83
GeneMark.hmm	195	0.87	0.89	0.84 ± 0.18	0.83
GENSCAN	195	0.95	0.90	0.91 ± 0.12	0.91
HMMgene	195	0.93	0.93	0.91 ± 0.13	0.91
MZEF	119	0.70	0.73	0.68 ± 0.21	0.66

- Overall performances are the best for HMMgene and GENSCAN.
- Some program's accuracy depends on the G+C content, except for HMMgene and GENSCAN, which use different parameters sets for different G+C contents.
- For almost all the tested programs, "medium" exons (70-200 nucleotides long), are most accurately predicted. Accuracy decrease for shorter and longer exons, except for HMMgene.
- Internal exons are much more likely to be correctly predicted (weakness of the start/stop codon detection).
- Initial and terminal exons are most likely to be missed completely.
- Only HMMgene and GENSCAN have reliable scores for exon prediction.

Accuracy of the different methods

Recently a new benchmark has been published by Makarov (2002), with similar results, but other predictors have been included.

Programs	S_n^a	S_p^a	S_n^b	S_p^b
HMMgene	97	91	93	93
GenScan		95	90	93
Geneid	86	83		
Genie	96	92	91	90
FGENES	89	77	86	88

^{a} Adh region of Drosophila.

^{b} 195 high-quality mammalian sequences (human, mouse, and rat).

Gene prediction limits

Existing predictors are for protein coding regions

- Non-coding areas are not detected (5’ and 3’ UTR)
- Non-coding RNA genes are missed

Predictions are for ”typical” genes

- Partial genes are often missed
- Training sets may be biased
- Atypical genes use other grammars

The end