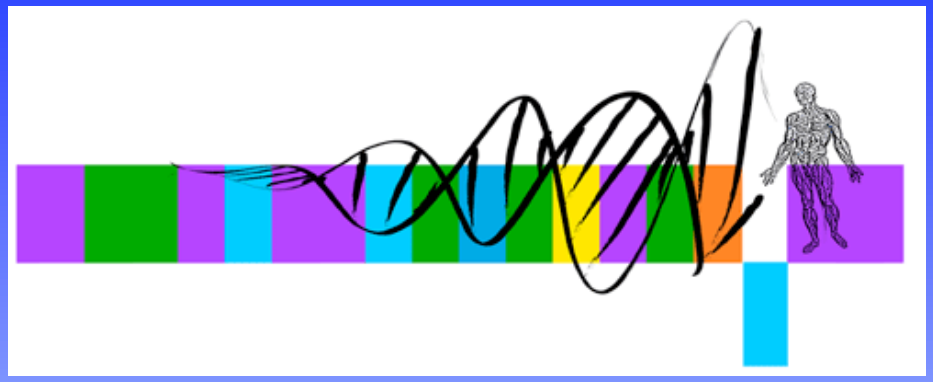

Bioinformatics for Biologists

Sequence Analysis: Part III. Gene Finding

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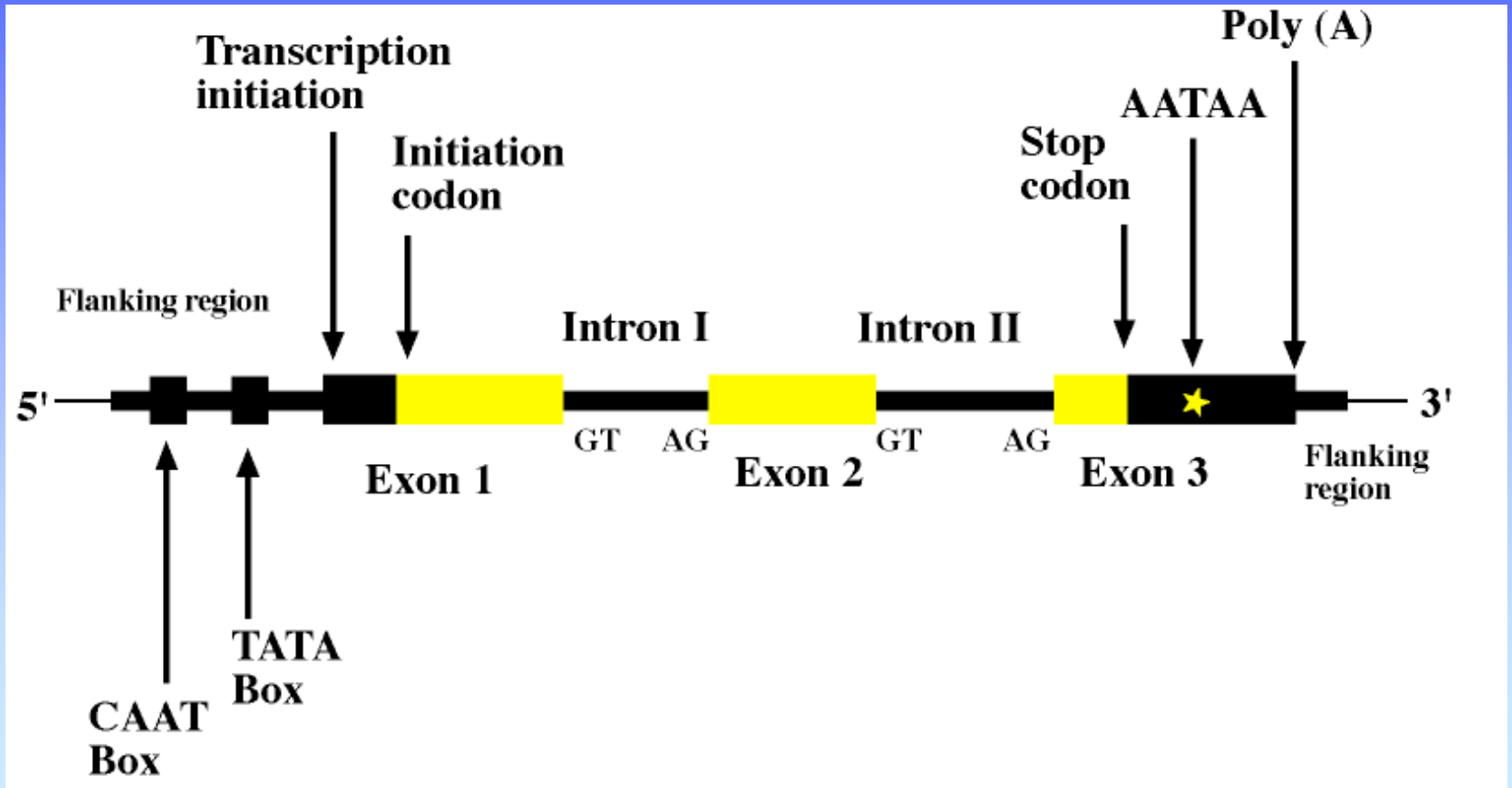
Interesting features in DNA

- ***Structure of genes*** - exons, introns, etc.
- ***Non-coding RNAs*** - including micro-RNAs and other small RNAs
- ***Promoter sites***
- ***Alternative splice forms***

Problem to Solve



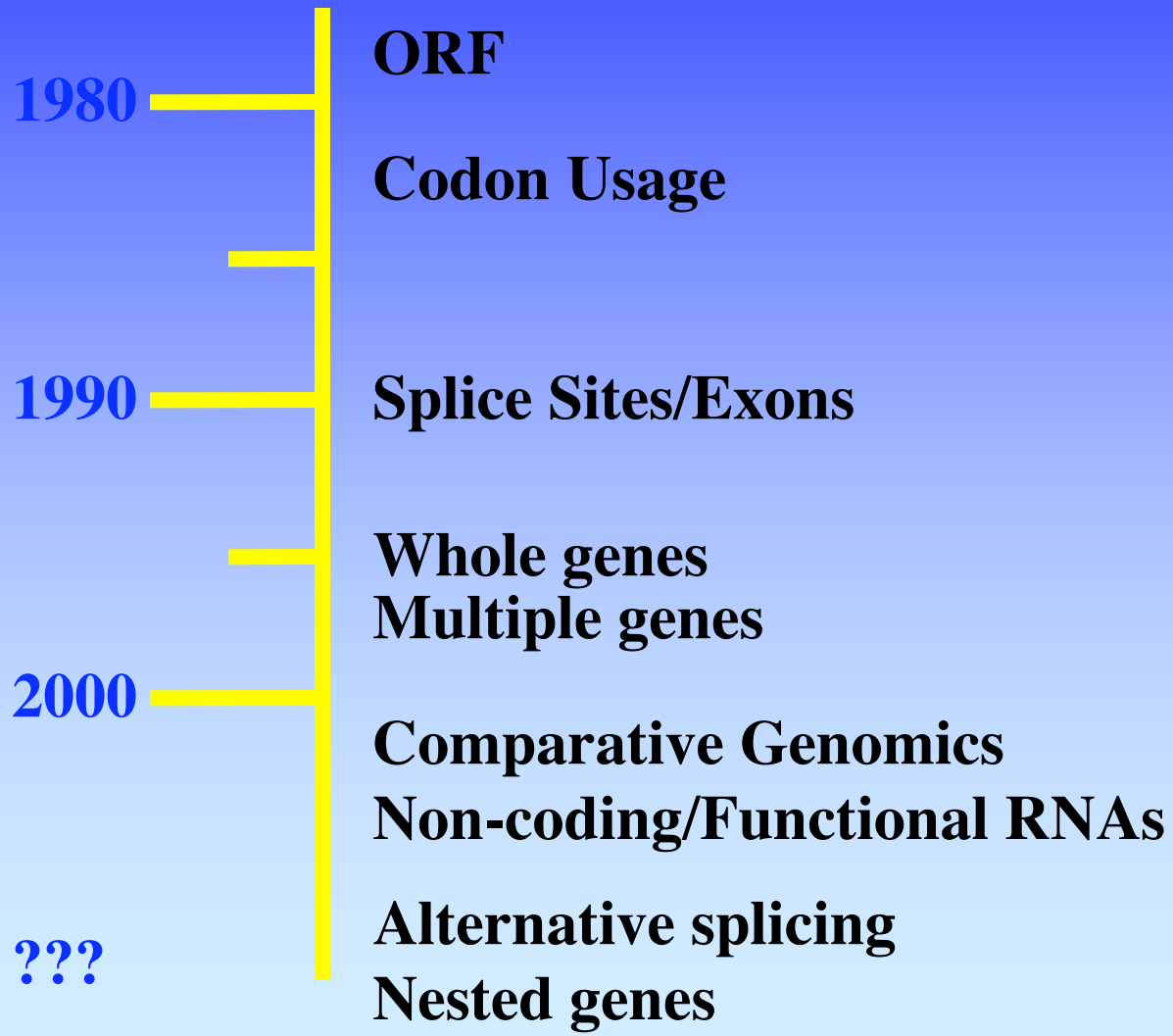
Types of Signals to Detect

- Transcriptional
  - TSS
  - TATA box
  - PolyA
- Translational
  - Kozak (CC A/G CCAUGG)
  - Termination codon (UAA, UAG, UGA)
- Splicing
  - Introns - GT……AG

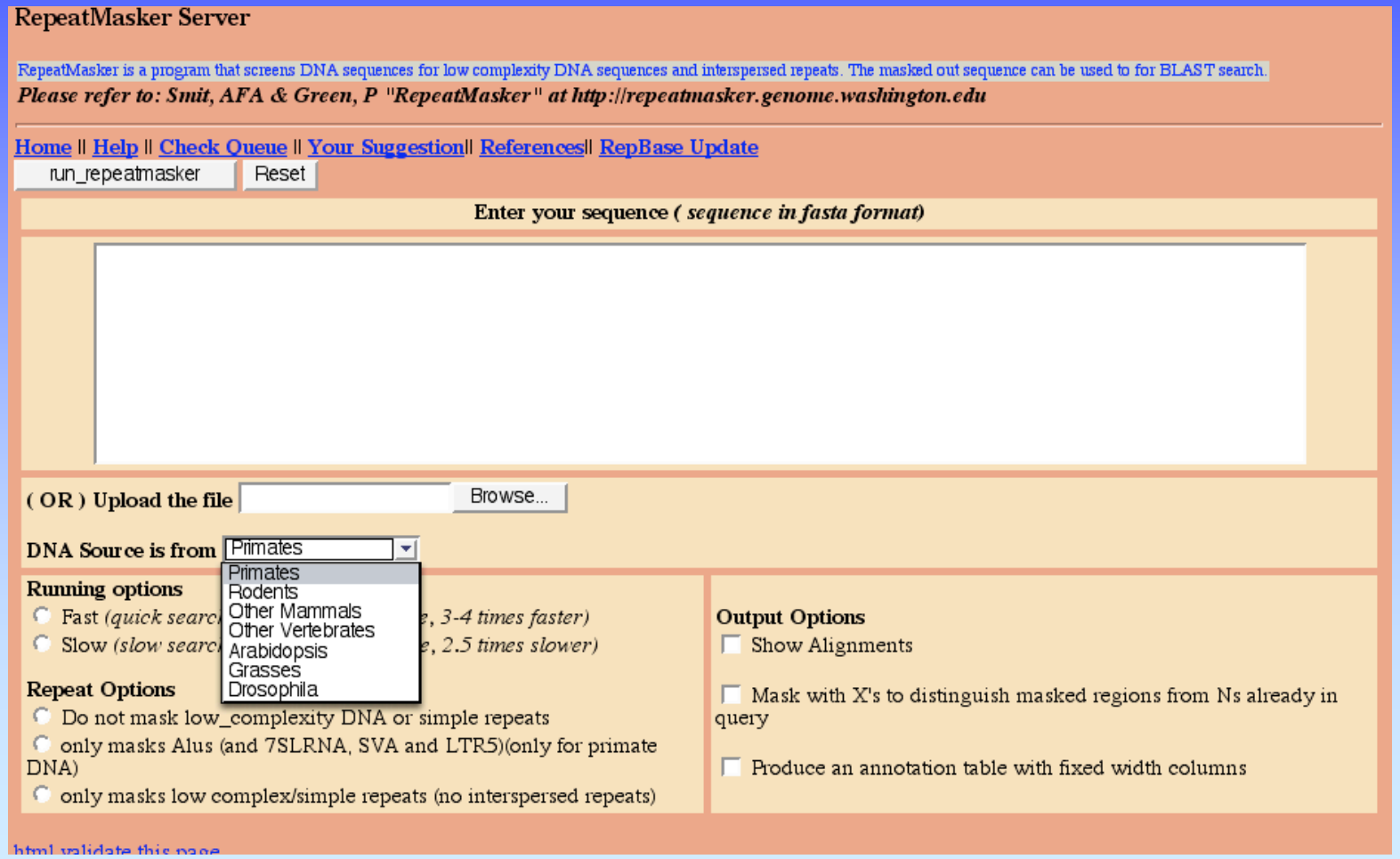
Gene Finding Strategies

- Content-based methods
  - codon usage, compositional complexity
- Site-based methods
  - presence or absence of specific pattern or sequence
- Comparative methods
  - determination based on homology

Evolution of Gene Finding Programs



RepeatMasker



RepeatMasker

Repeat sequence:

SW score	perc div.	perc del.	perc ins.	query sequence	position in query begin	end	(left)		matching repeat	class/family	position in repeat begin	end	(left)	ID
2117	3.9	0.0	1.2	myseq	7	263	(8487)	+	AluY	SINE/Alu	58	311	(0)	1
7658	20.9	6.8	5.7	myseq	2193	2517	(6233)	C	L1MDa	LINE/L1	(1460)	5080	4760	2
2516	4.9	2.0	0.0	myseq	2518	2822	(5928)	+	AluY	SINE/Alu	1	311	(0)	3
7658	20.9	6.8	5.7	myseq	2823	4135	(4615)	C	L1MDa	LINE/L1	(1780)	4760	3462	2
5685	6.2	0.8	0.0	myseq	4136	4864	(3886)	+	L1PA10	LINE/L1	5417	6151	(17)	4
7658	20.9	6.8	5.7	myseq	4865	5181	(3569)	C	L1MDa	LINE/L1	(3078)	3462	3150	2
2130	11.2	3.0	0.7	myseq	5182	5303	(3447)	+	AluSq	SINE/Alu	1	119	(194)	5
351	0.0	0.0	0.0	myseq	5304	5342	(3408)	+	(TAAA)n	Simple_repeat	2	40	(0)	6
2130	11.2	3.0	0.7	myseq	5343	5514	(3236)	+	AluSq	SINE/Alu	119	302	(11)	5
2593	6.6	2.8	0.0	myseq	5525	5886	(2864)	+	L1PA13	LINE/L1	5792	6163	(0)	7
7658	20.9	6.8	5.7	myseq	5887	6390	(2360)	C	L1MDa	LINE/L1	(3398)	3142	2645	2
2092	9.6	1.0	3.6	myseq	6391	6693	(2057)	C	AluSc	SINE/Alu	(13)	296	2	9
7658	20.9	6.8	5.7	myseq	6694	8738	(12)	C	L1MDa	LINE/L1	(3895)	2645	491	2

RepeatMasker

Summary:

Total length: 8750 bp

GC level: 35.61%

Bases masked: 6803 bp (77.75%)

	number of elements*	length occupied	percentage of sequence
SINEs:	4	1159 bp	13.25 %
ALUs	4	1159 bp	13.25 %
MIRs	0	0 bp	0.00 %
LINEs:	3	5605 bp	64.06 %
LINE1	3	5605 bp	64.06 %
LINE2	0	0 bp	0.00 %
L3/CR1	0	0 bp	0.00 %
LTR elements:	0	0 bp	0.00 %
MaLRs	0	0 bp	0.00 %
ERVL	0	0 bp	0.00 %
ERV_classI	0	0 bp	0.00 %
ERV_classII	0	0 bp	0.00 %
DNA elements:	0	0 bp	0.00 %
MER1_type	0	0 bp	0.00 %
MER2_type	0	0 bp	0.00 %
Unclassified:	0	0 bp	0.00 %
Total interspersed repeats:		6764 bp	77.30 %
Small RNA:	0	0 bp	0.00 %
Satellites:	0	0 bp	0.00 %
Simple repeats:	1	39 bp	0.45 %
Low complexity:	0	0 bp	0.00 %

Coding Measures

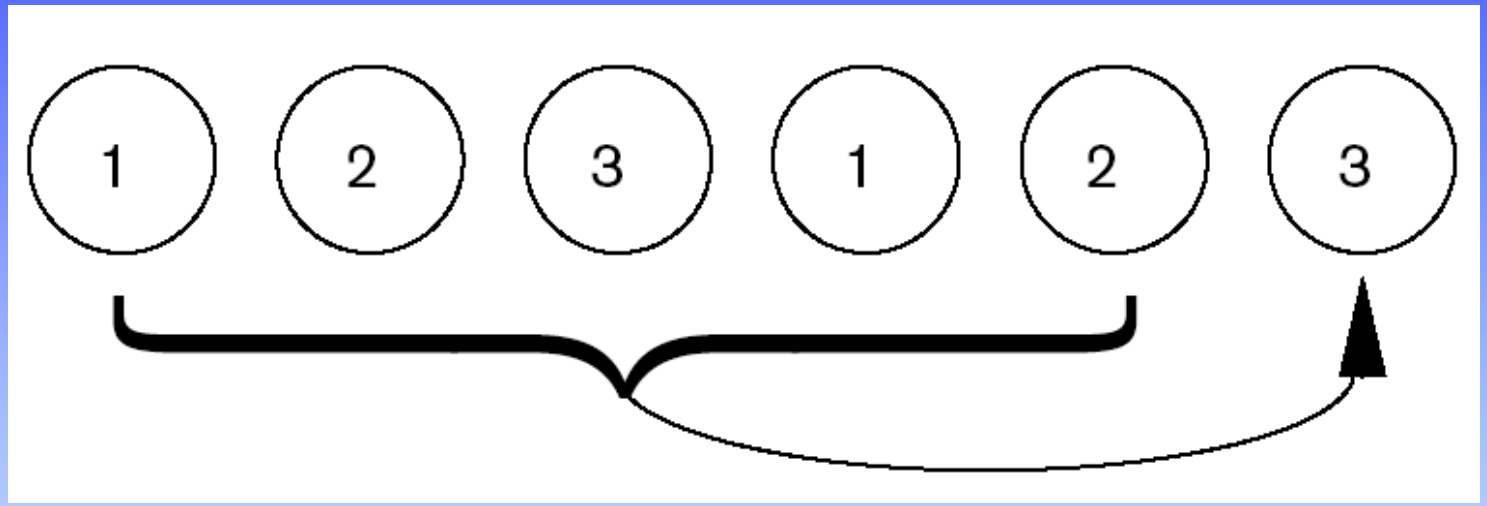
- Look at frequencies of codons (e.g. redundancy of genetic code; Leucine = UUA, UUG, CUU, CUC, CUA, CUG)

- 6-tuple or hexamer approach

  ACCTCG TACTCG GCCCTC

  Thr Ser Tyr Ser Ala Leu

Fifth Order Markov Models

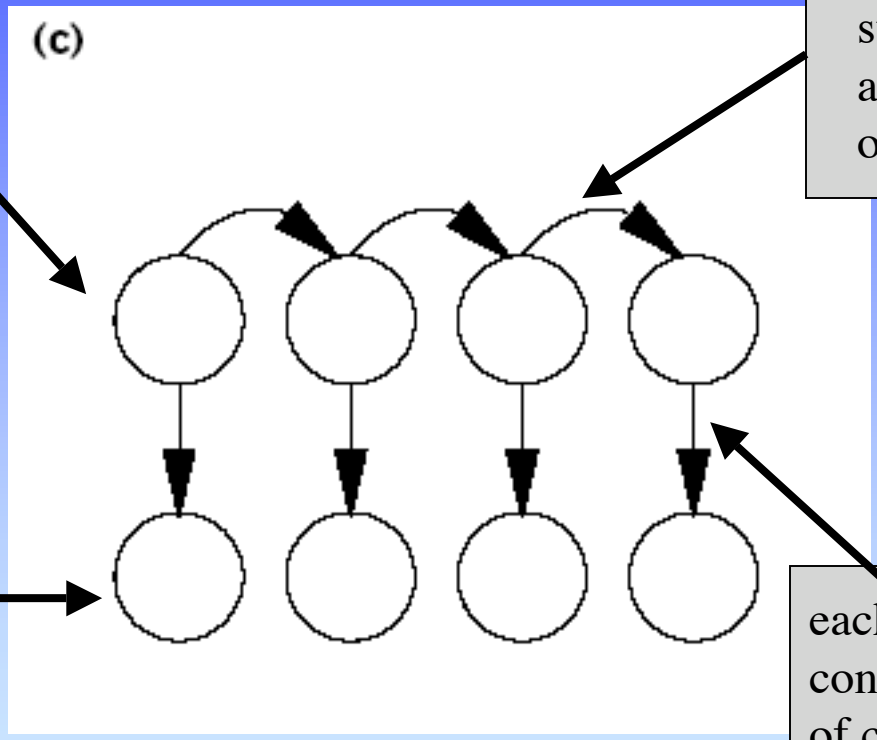


Periodic fifth order Markov model. Circles represent consecutive DNA bases, numbers indicate codon position, and the arrows indicate that the next base is generated conditionally on the previous five and on the codon position.

Burge and Karlin, Current Opinions in Structural Biology 1998, 8:346-354.

Hidden Markov Models

unobserved or "hidden" states (e.g. coding or noncoding)



states generated according to a first order Markov chain

observable DNA bases

each base generated conditionally on identity of corresponding hidden state

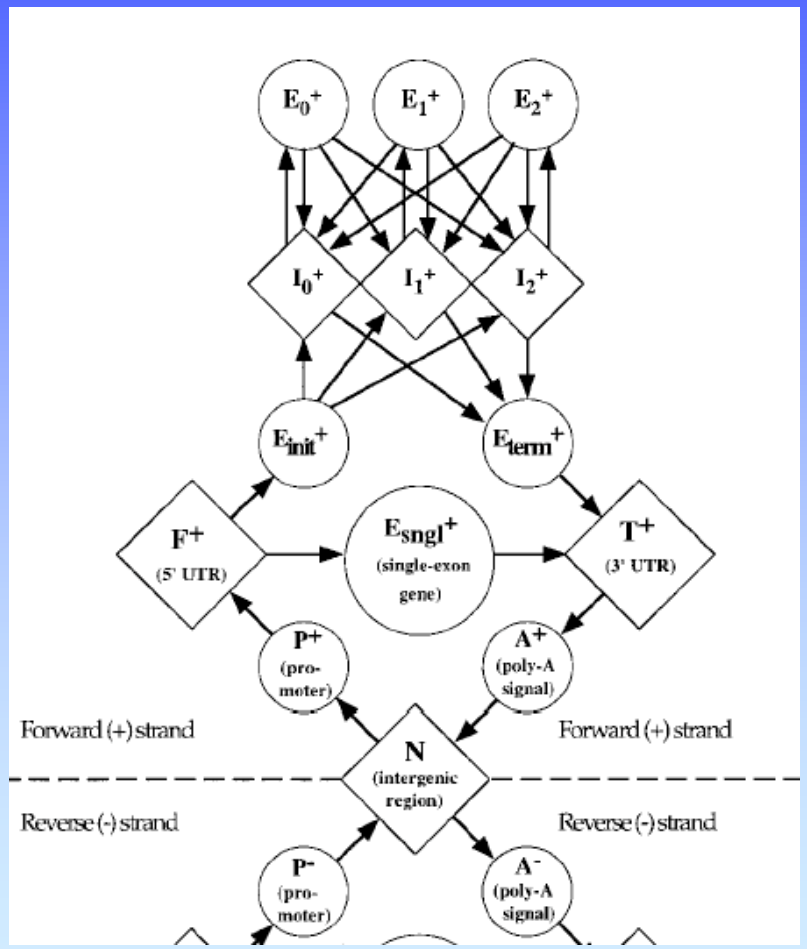
Burge and Karlin, Current Opinions in Structural Biology 1998, 8:346-354.

GENSCAN

- MM - prob for a given nuc to occur at position *p* depends on nuc occupying previous *k* positions
- Generalized Hidden Markov Model (GHMM)
- Optimize module performing signal recognition
- Incorporates influence of C+G content
- Considers gene models on both strands
- Can identify multiple genes

Burge and Karlin, JMB:268:78-94, 1997

GENSCAN



Burge and Karlin,
JMB:268:78-94, 1997

Gene Finding Programs

- FGENESH - Softberry
- GeneID - Barcelona
- GeneMark HMM - Georgia Tech
- Genie - UCSC & LBNL
- Genscan - Stanford and MIT
- GenomeScan - MIT
- MZEF/First exon - Cold Spring Harbor
- Twinscan - WU

HMR195 Test Set

- 103 human, 82 mouse, 10 rat sequences
- Sequence new since August, 1997
- Genomic sequences containing exactly one gene
- No mRNA sequences, pseudogenes or alternatively spliced genes
- The mean length of sequences is 7,096 bp

Rogic, Mackworth and Ouellette, Genome Research 11:817-832, 2001

HMR195 Test Set (con't)

- 43 single-exon genes; 152 multi-exon genes
- Average number of exons per gene is 4.86
- Mean exon length = 208 bp,
  mean intron length = 678 bp,
  mean coding length per gene = 1,015 bp
  (~330 aa)
- Coding sequence 14%, intronic sequence 46% and intergenic DNA 40%.

Rogic, Mackworth and Ouellette, Genome Research 11:817-832, 2001

Definitions

- ***Sensitivity:*** the proportion of true sites(e.g., exons or donor splice sites) which are correctly predicted = TP/(TP + FN)

- ***Specificity:*** the proportion of predicted sites which are correct = TP/(TP + FP)

Program Comparisons Results

- Genscan and HMMgene had reliable scores for exons
- Nucleotide Sn = .95 for Genscan and .93 for HMMgene.
- Sp = .90 and .93, respectively
- Accuracy dependent on G+C content

Rogic, Mackworth and Ouellette, Genome Research 11:817-832, 2001

Table 2. Accuracy versus Signal Type

Programs	Signal type: start codon (195)	acceptor site (753)	donor site (753)	stop codon (195)
FGENES	0.67 (0.63)	0.80 (0.77)	0.85 (0.82)	0.75 (0.72)
GeneMark.hmm	0.46 (0.60)	0.81 (0.75)	0.82 (0.78)	0.57 (0.64)
Genie	0.56 (0.57)	0.77 (0.82)	0.78 (0.83)	0.72 (0.73)
Genscan	0.61 (0.78)	0.87 (0.80)	0.90 (0.84)	0.76 (0.86)
HMMgene	0.75 (0.78)	0.81 (0.85)	0.83 (0.87)	0.78 (0.81)
Morgan	0.43 (0.43)	0.66 (0.57)	0.65 (0.56)	0.39 (0.39)
MZEF	—	0.59 (0.65)	0.66 (0.73)	—

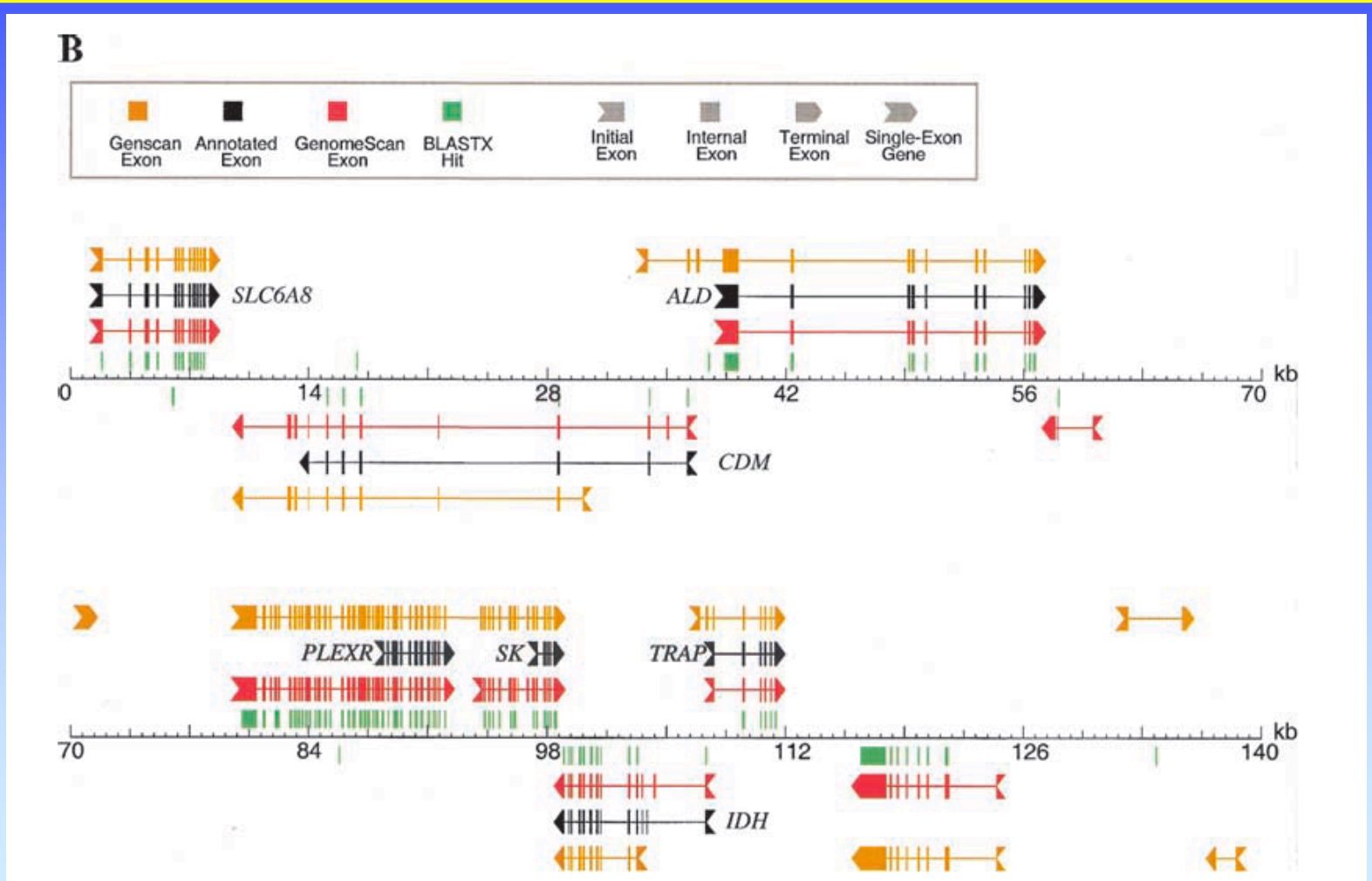
For each program, the proportion of actual signals identified correctly (the upper number) and the proportion of predicted signals that are correct (the lower number) are averaged over all signals belonging to a particular type. The number in parenthesis in the header of each column represents the number of signals of each type in the HMR195 dataset.

GenomeScan

- Combines exon-intron and splice signal models with similarity to know proteins
- Used to identify genes in human draft sequence
- Uses GENSCAN and BLASTX

Yeh, Lim, and Burge, Genome Research 11:803-816, 2001.

GenomeScan



Yeh, Lim, and Burge, Genome Research 11:803-816, 2001.

GenomeScan

Table 4. Summary of GenomeScan-predicted Genes and Partial Genes in the Human Genome

	Type of predicted gene						
	Complete genes (>2 exons)			Partial genes		All genes (partial + complete)	
Similarity category	No. of genes	No. of exons/gene	No. of aa/gene	No. of genes	No. of exons/gene	No. of genes	% of all predicted genes
Known (cDNA)	5698	9.6	496	8901	4.9	16040	41.5
Protein + EST	4502	8.8	510	6537	5.5	12546	32.5
Proteins only	2767	5.2	303	4600	3.1	10061	26.0
All	12967	8.4	460	20038	4.7	38647	100.0

Genes were predicted in the September 2000 GoldenPath human genome sequence as described in Methods. Predicted coding sequences (CDS) were first compared to cDNAs in the RefSeq cDNA database (September 2000) using `BLASTN`; those which had a hit at least 100 bp long with at least 98% identity are listed as "known". The remaining predicted coding sequences were searched against dbEST (September 2000 release) using `BLASTN`; those which had a hit at least 100 bp long with at least 97% identity are listed as "Protein + EST". All other predicted genes are categorized as "Protein only" because all `GenomeScan`-predicted genes have at least modest similarity to a known protein. Statistics are listed separately for predicted partial genes and predicted complete genes with at least three exons; the category "all genes" includes these two groups as well as predicted 1- and 2-exon genes.

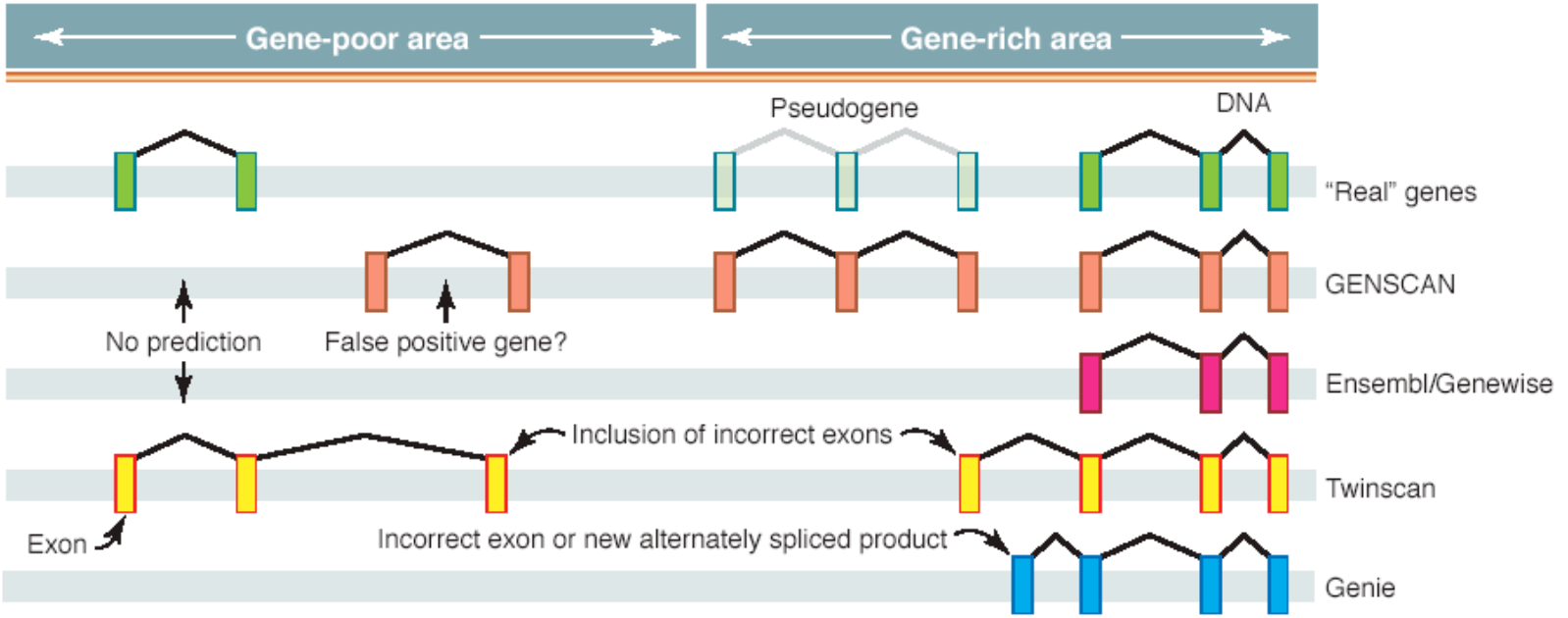
Yeh, Lim, and Burge, Genome Research 11:803-816, 2001.

Other Approaches

- Use microarrays to identify expressed genes based on the coexpression of sets of adjacent exons as predicted by GENSCAN (Shoemaker, et al, Nature 409:922-927, 2001)

- RT-PCR with radio-labeled primers targeted to pairs of adjacent predicted exons, followed by sequencing of the amplified product (Das, Burge et al, Genomics. 77:71-8, 2001)

E. Pennisi, Science 301:1040, 2003



Never perfect. No program calls all genes correctly. Some see genes (shown here as coding regions, or exons, connected by bent lines) where there are none; some miss a gene altogether; and some don't put all the gene's parts in the right places.

Comparative Genomics

Cliften P, et al. Finding functional features in Saccharomyces genomes by phylogenetic footprinting. *Science*. *301:71-6, 2003*.

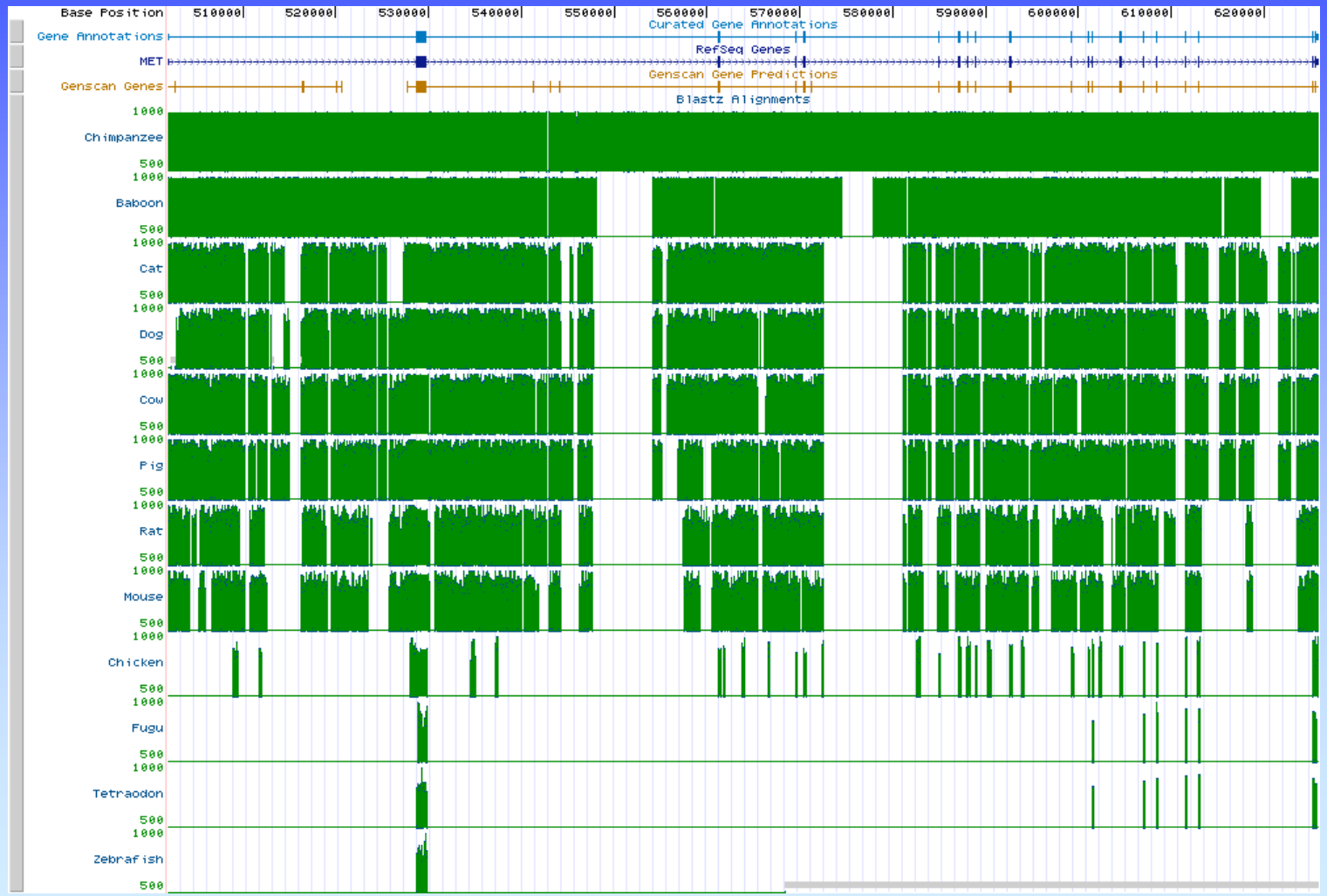
Kellis M, et al Sequencing and comparison of yeast species to identify genes and regulatory elements. *Nature 423:241-54, 2003*.

Thomas JW, et al. Comparative analyses of multi-species sequences from targeted genomic regions. *Nature 424:788-93, 2003*

Some other useful Tools

- GeneWise - compares protein sequence to genomic DNA sequence, allowing for introns and frameshifting errors
- Pipmaker/Multipipmaker - long alignments of two or multiple genomic regions (local)
- VISTA - whole genome alignments - human, mouse, rat (global)
- UCSC Genome Browser

UCSC Genome Browser



Future Challenges

- Alternative Splicing
- Gene products functioning at RNA level
- Nested genes
- 5' end of genes
- Other unusual characteristics