Gene Structure & Gene Finding Part II

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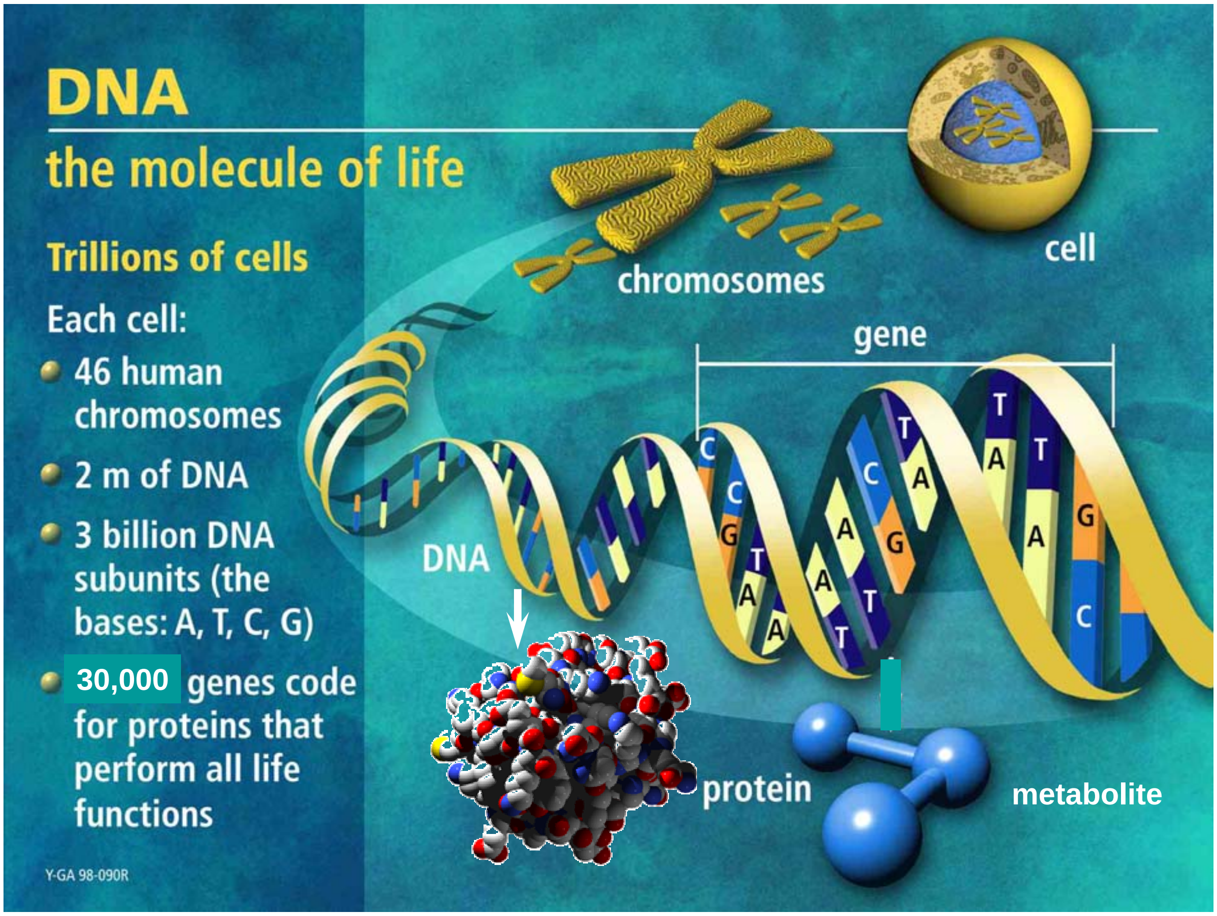


DNA

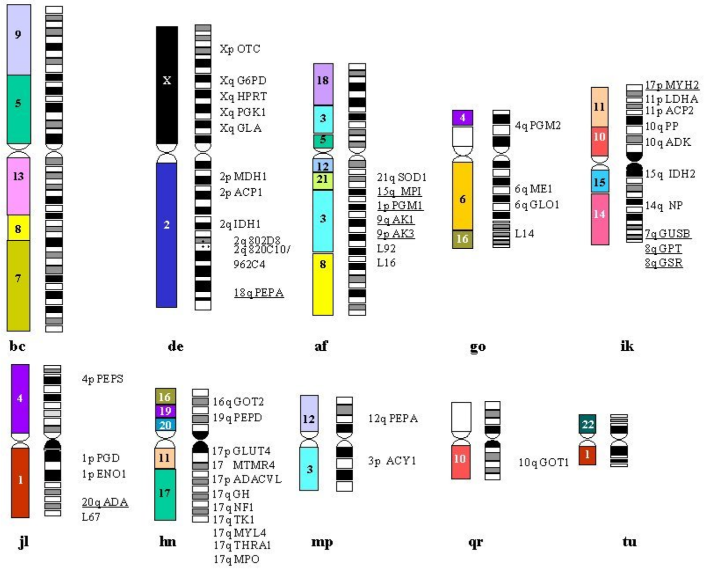
the molecule of life

Trillions of cells

Each cell:

- 46 human chromosomes
- 2 m of DNA
- 3 billion DNA subunits (the bases: A, T, C, G)
- 30,000 genes code for proteins that perform all life functions

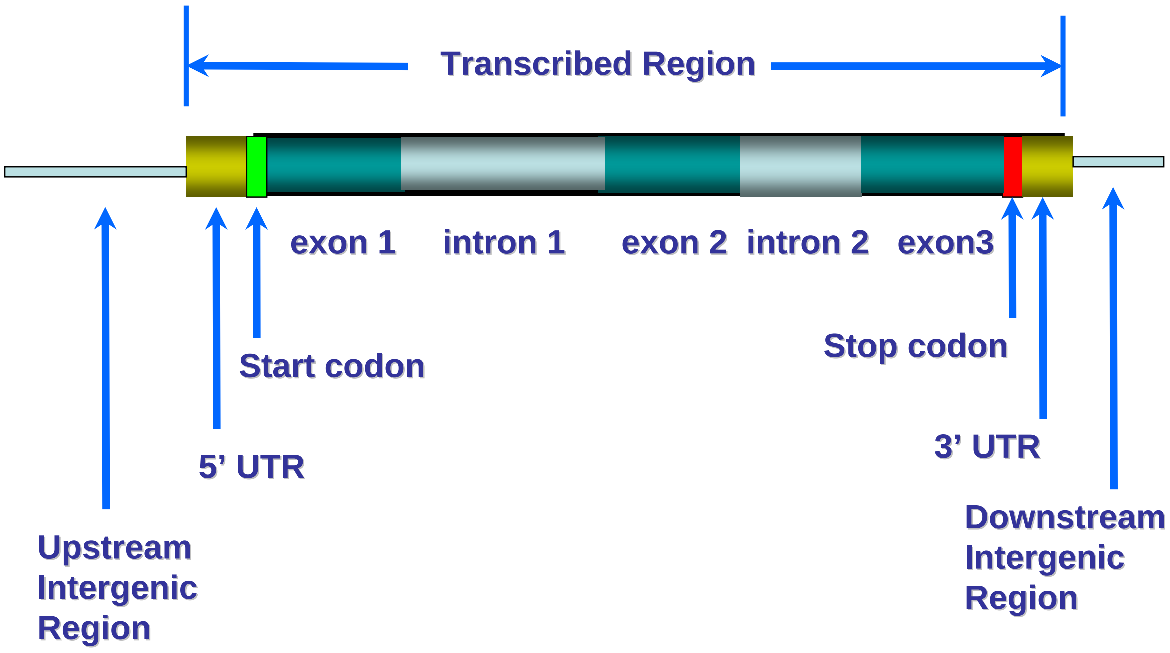
Gene Finding in Eukaryotes



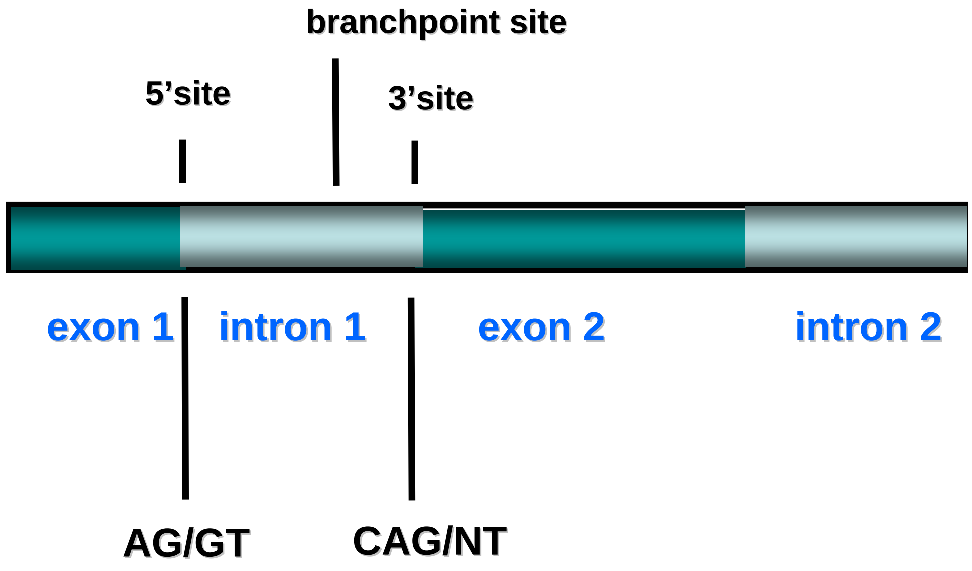
Eukaryotes

- Complex gene structure
- Large genomes (0.1 to 10 billion bp)
- Exons and Introns (interrupted)
- Low coding density (<30%)
  - 3% in humans, 25% in Fugu, 60% in yeast
- Alternate splicing (40-60% of all genes)
- High abundance of repeat sequence (50% in humans) and pseudo genes
- Nested genes: overlapping on same or opposite strand or inside an intron

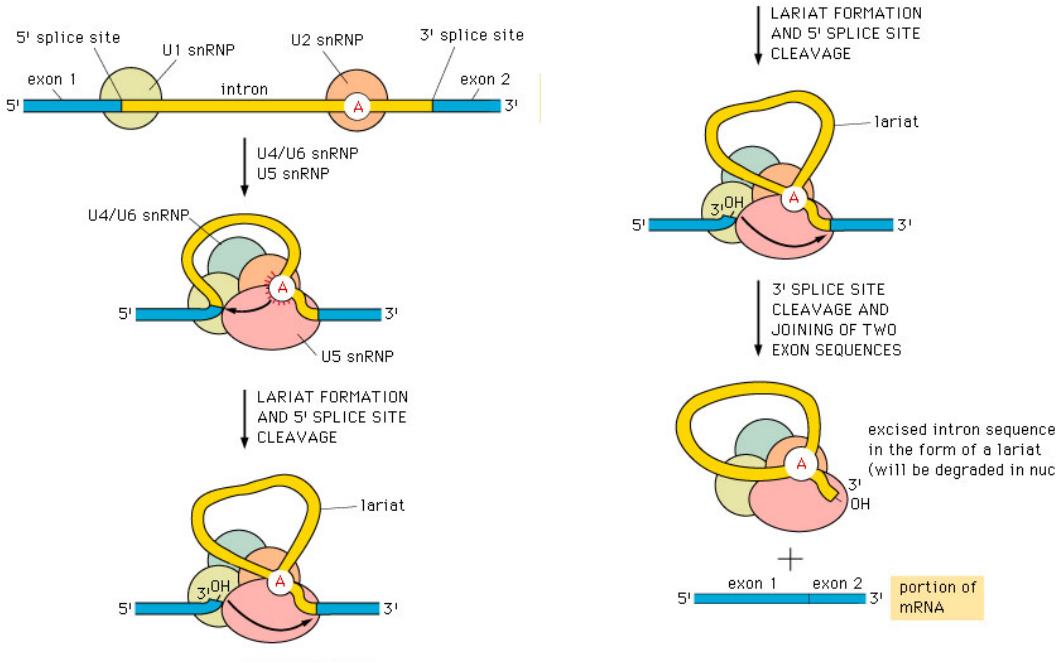
Eukaryotic Gene Structure



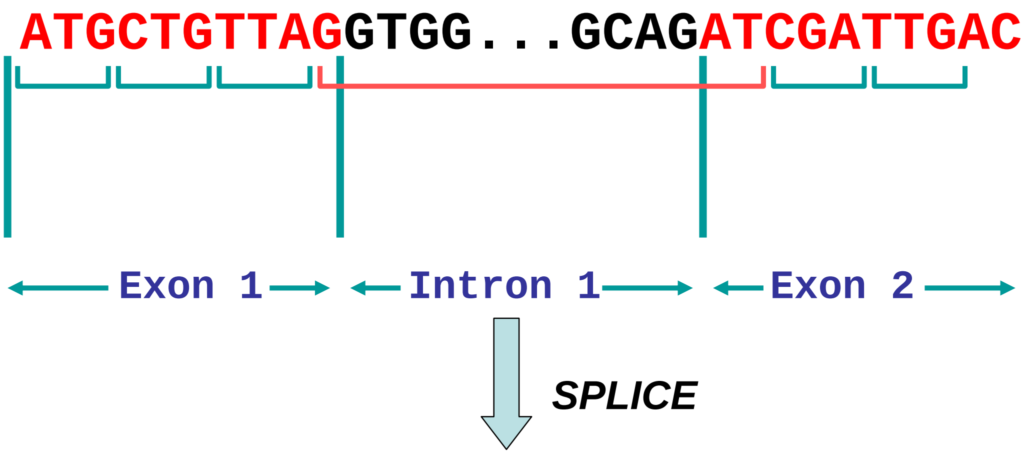
Eukaryotic Gene Structure



RNA Splicing



Exon/Intron Structure (Detail)



ATGCTGTTAGATCGATTGAC

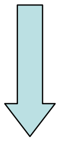
Intron Phase

- A codon can be interrupted by an intron in one of three places

Phase 0: ATGATTGTCAG...CAGTAC

Phase 1: ATGATGTCAG...CAGTTAC

Phase 2: ATGAGTCAG...CAGTTTAC



SPLICE

AGTATTTAC

Repetitive DNA

- Moderately Repetitive DNA
  - Tandem gene families (250 copies of rRNA, 500-1000 tRNA gene copies)
  - Pseudogenes (dead genes)
  - Short interspersed elements (SINEs)
    - 200-300 bp long, 100,000+ copies, scattered
    - Alu repeats are good examples
  - Long interspersed elements (LINEs)
    - 1000-5000 bp long
    - 10 - 10,000 copies per genome

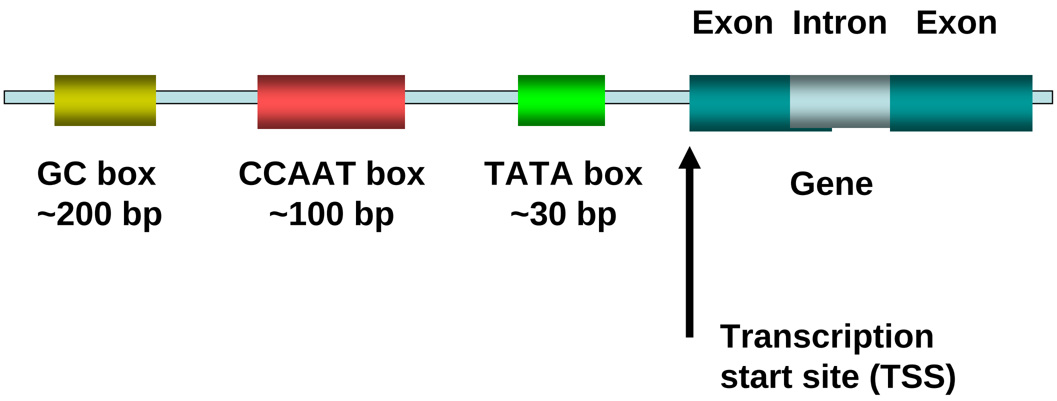
Repetitive DNA

- Highly Repetitive DNA
  - Minisatellite DNA
    - repeats of 14-500 bp stretching for ~2 kb
    - many different types scattered thru genome
  - Microsatellite DNA
    - repeats of 5-13 bp stretching for 100’s of kb
    - mostly found around centromere
  - Telomeres
    - highly conserved 6 bp repeat (TTAGGG)
    - 250-1000 repeats at end of each chromosome

Key Eukaryotic Gene Signals

- Pol II RNA promoter elements
  - Cap and CCAAT region
  - GC and TATA region
- Kozak sequence (Ribosome binding site-RBS)
- Splice donor, acceptor and lariat signals
- Termination signal
- Polyadenylation signal

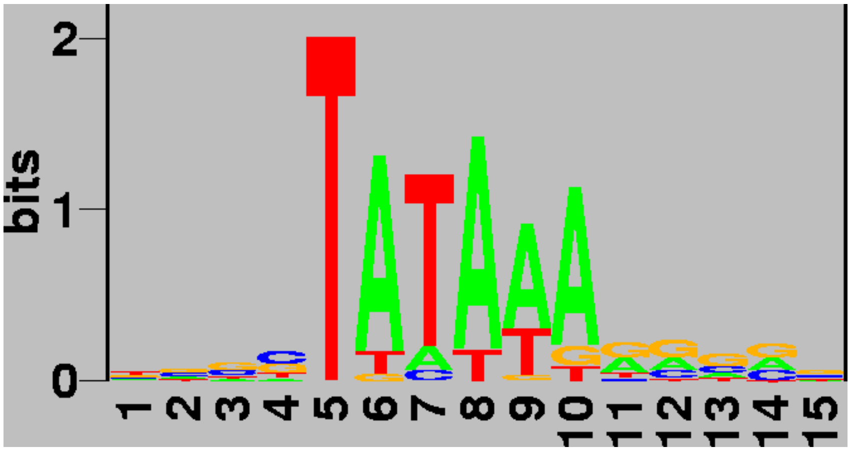
Pol II Promoter Elements



Pol II Promoter Elements

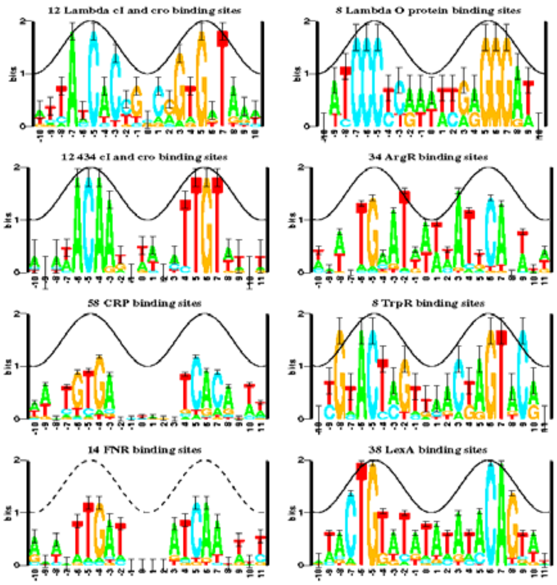
- Cap Region/Signal
  - n C A G T n G
- TATA box (~ 25 bp upstream)
  - T A T A A A n G C C C
- CCAAT box (~100 bp upstream)
  - T A G C C A A T G
- GC box (~200 bp upstream)
  - A T A G G C G nGA

Pol II Promoter Elements



TATA box is found in ~70% of promoters

WebLogos


12 Lambda cI and cro binding sites

8 Lambda O protein binding sites

12 434 cI and cro binding sites

34 ArgR binding sites

58 CRP binding sites

8 TrpR binding sites

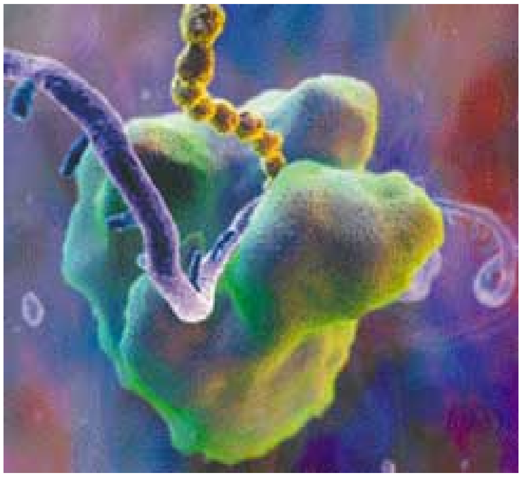
14 FNR binding sites

38 LexA binding sites

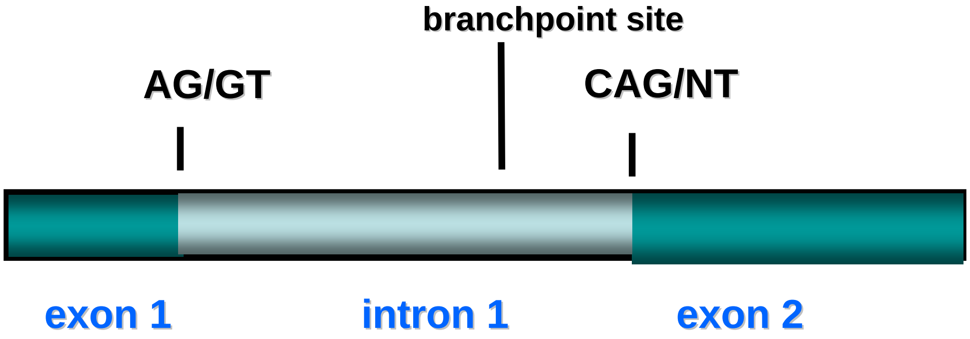
http://www.bio.cam.ac.uk/cgi-bin/seqlogo/logo.cgi

Kozak (RBS) Sequence

-7	-6	-5	-4	-3	-2	-1	0	1	2	3
A	G	C	C	A	C	C	A	T	G	G



Splice Signals



branchpoint site

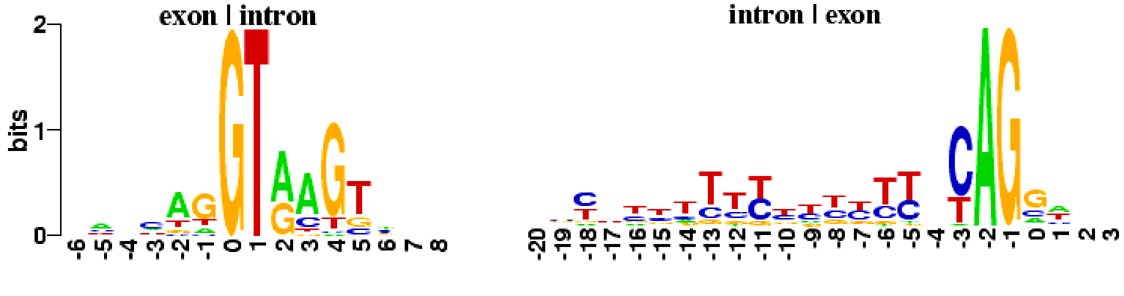
AG/GT

CAG/NT

exon 1

intron 1

exon 2



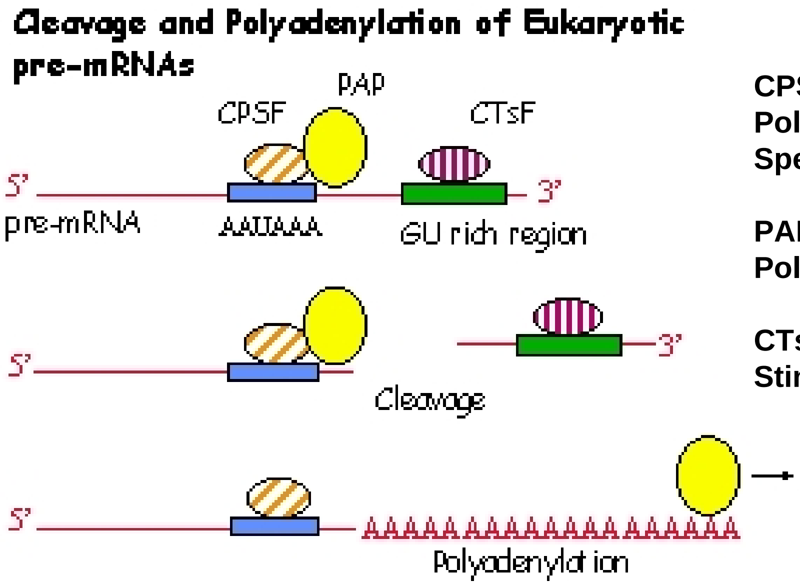
Splice Sites

- Not all splice sites are real
- ~0.5% of splice sites are non-canonical (i.e. the intron is not GT...AG)
- It is estimated that 5%of human genes may have non-canonical splice sites
- ~50% of higher eukaryotes are alternately spliced (different exons are brought together)

Miscellaneous Signals

- Polyadenylation signal
  - AATAAA or ATTAAA
  - Located 20 bp upstream of poly-A cleavage site
- Termination Signal
  - AGTGTTCA
  - Located ~30 bp downstream of poly-A cleavage site

Polyadenylation



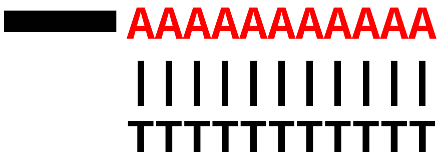
CPSF – Cleavage & Polyadenylation Specificity Factor

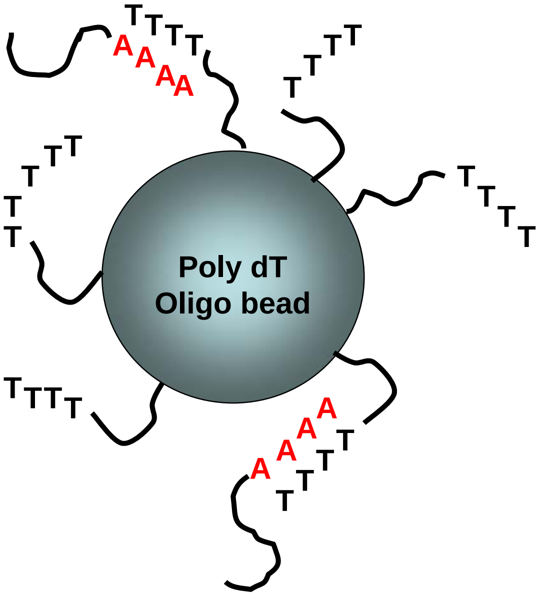
PAP – Poly-A Polymerase

CTsF – Cleavage Stimulation Factor

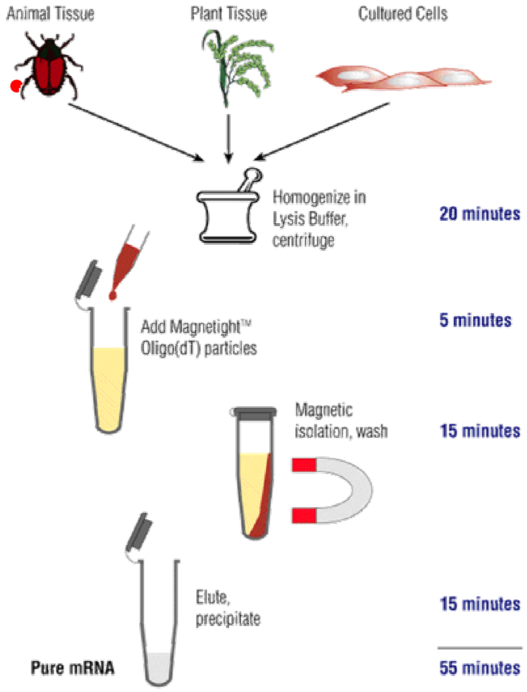
Why Polyadenylation is Really Useful

Complementary Base Pairing



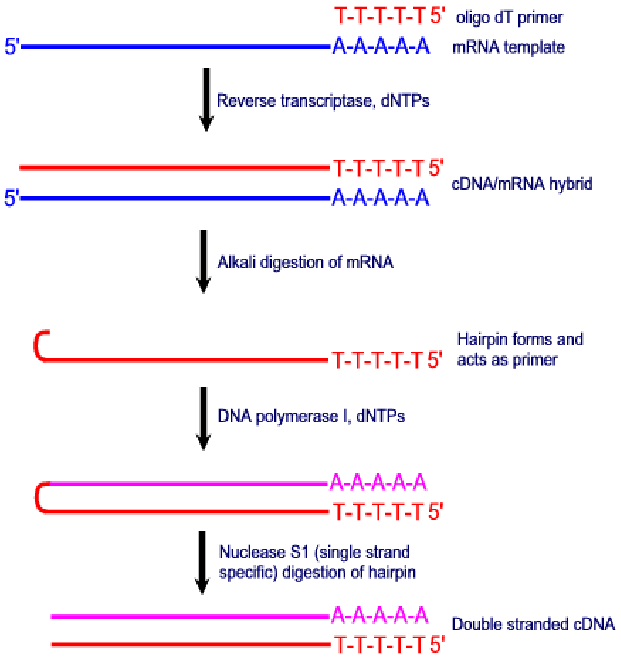


mRNA isolation



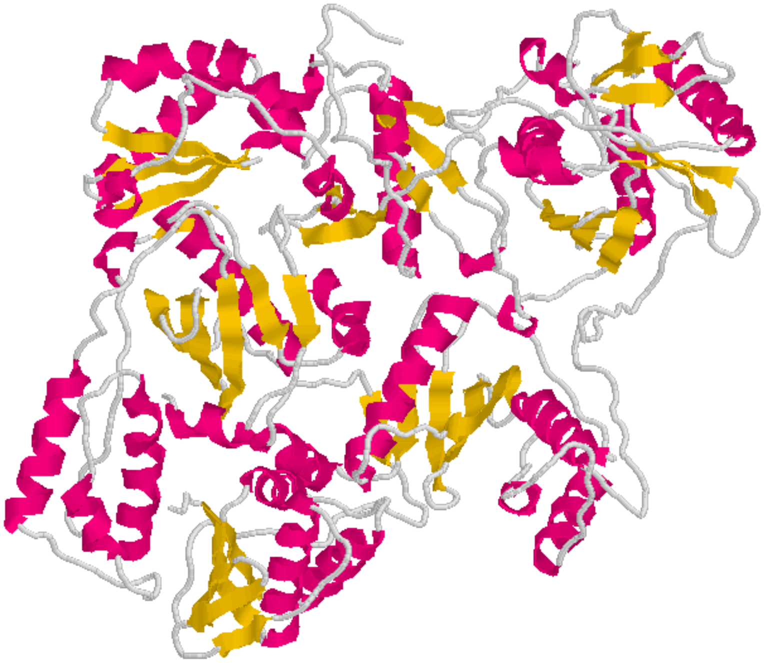
- Cell or tissue sample is ground up and lysed with chemicals to release mRNA
- Oligo(dT) beads are added and incubated with mixture to allow A-T annealing
- Pull down beads with magnet and pull off mRNA

Making cDNA from mRNA



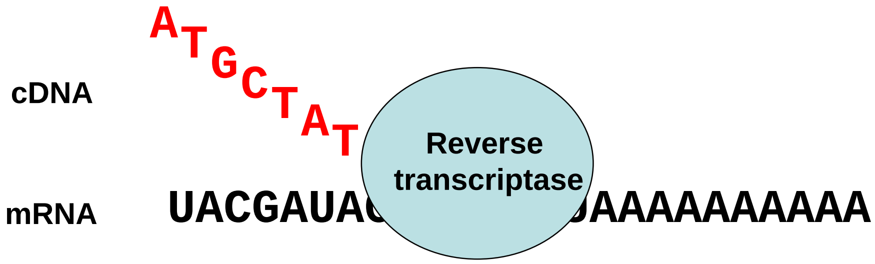
- cDNA (i.e. complementary DNA) is a single-stranded DNA segment whose sequence is complementary to that of messenger RNA (mRNA)
- Synthesized by reverse transcriptase

Reverse Transcriptase



Finding Eukaryotic Genes Experimentally

- Convert the spliced mRNA into cDNA



- Only expressed genes or expressed sequence tags (EST's) are seen
- Saves on sequencing effort (97%)

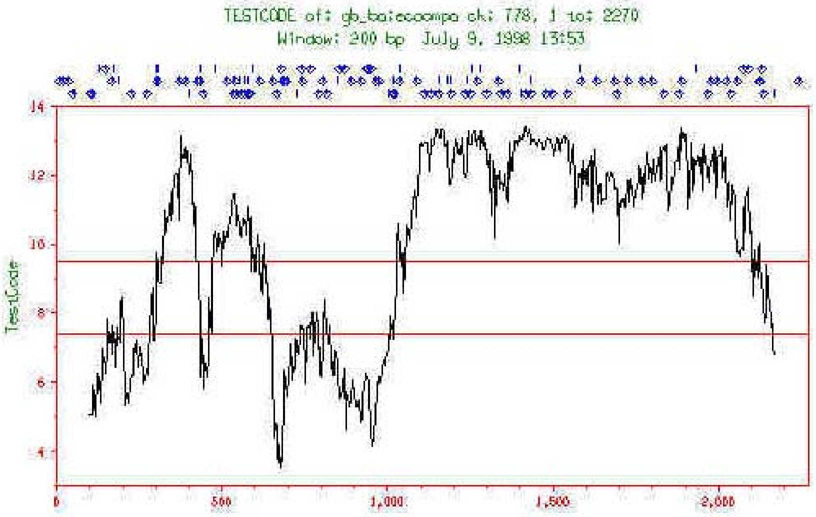
Finding Eukaryotic Genes Computationally

- Content-based Methods
  - GC content, hexamer repeats, composition statistics, codon frequencies
- Site-based Methods
  - donor sites, acceptor sites, promoter sites, start/stop codons, polyA signals, lengths
- Comparative Methods
  - sequence homology, EST searches
- Combined Methods

Content-Based Methods

- CpG islands
  - High GC content in 5’ ends of genes
- Codon Bias
  - Some codons are strongly preferred in coding regions, others are not
- Positional Bias
  - 3rd base tends to be G/C rich in coding regions
- Ficketts Method
  - looks for unequal base composition in different clusters of i, i+3, i+6 bases - TestCode graph

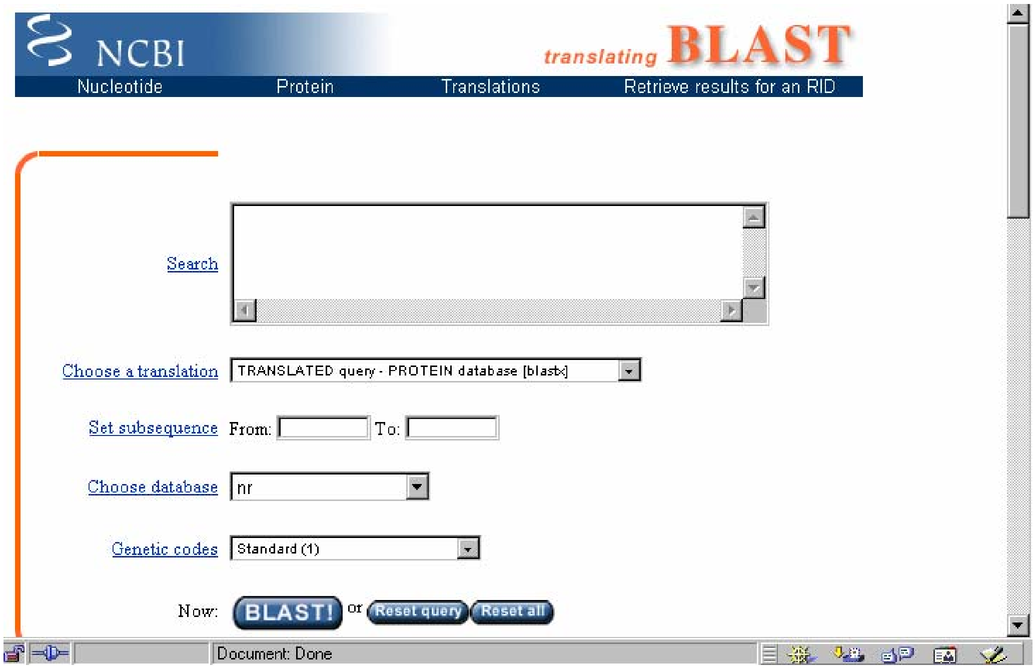
TestCode Plot



Comparative Methods

- Do a BLASTX search of all 6 reading frames against known proteins in GenBank
- Assumes that the organism under study has genes that are homologous to known genes (used to be a problem, in 2001 analysis of chr. 22 only 50% of genes were similar to known proteins)
- BLAST against EST database (finds possible or probable 3' end of cDNAs)

BLASTX



Search

Choose a translation: TRANSLATED query - PROTEIN database [blastx]

Set subsequence From: To:

Choose database: nr

Genetic codes: Standard (1)

Now:

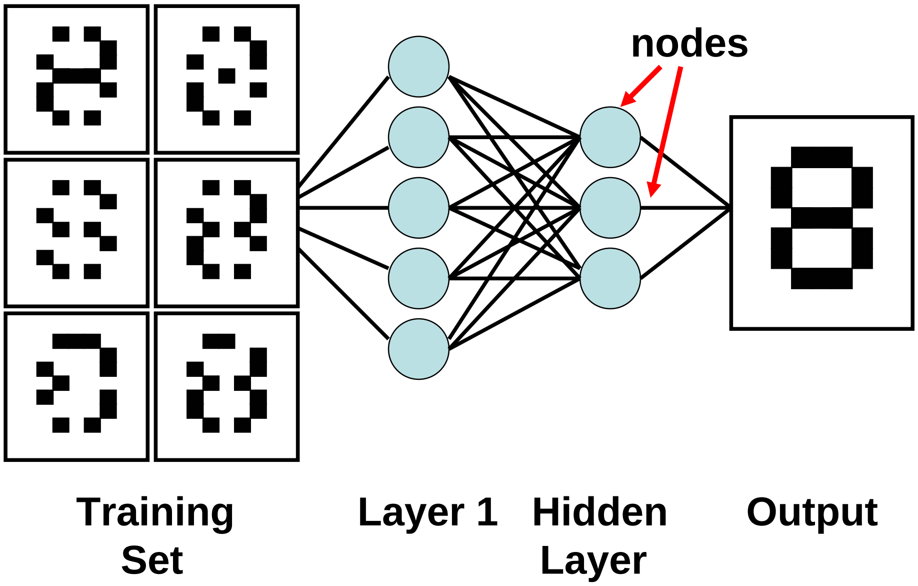
Site-Based Methods

- Based on identifying gene signals (promoter elements, splice sites, start/stop codons, polyA sites, etc.)
- Wide range of methods
  - consensus sequences
  - weight matrices
  - neural networks
  - decision trees
  - hidden markov models (HMMs)

Neural Networks

- Automated method for classification or pattern recognition
- First described in detail in 1986
- Mimic the way the brain works
- Use Matrix Algebra in calculations
- Require “training” on validated data
- *Garbage in = Garbage out*

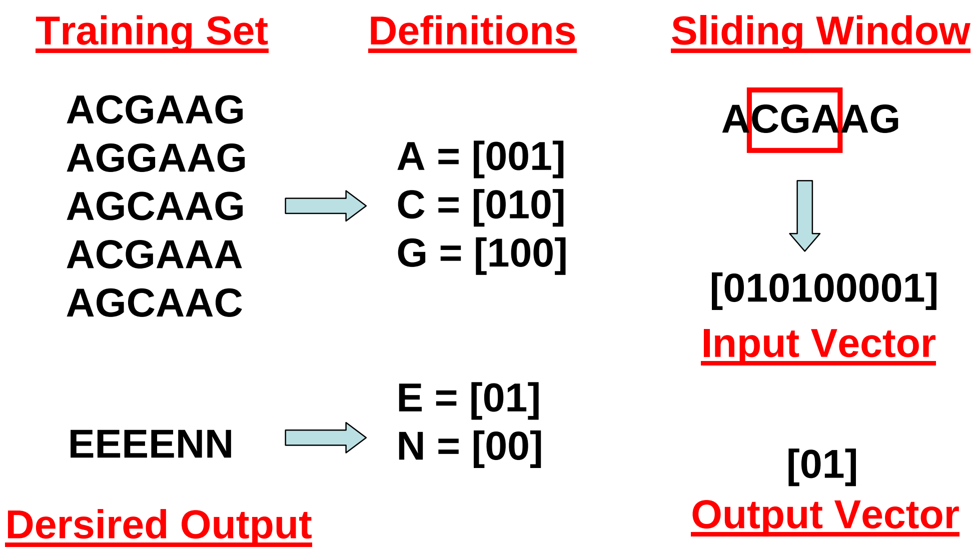
Neural Networks



Neural Network Applications

- Used in Intron/Exon Finding
- Used in Secondary Structure Prediction
- Used in Membrane Helix Prediction
- Used in Phosphorylation Site Prediction
- Used in Glycosylation Site Prediction
- Used in Splice Site Prediction
- Used in Signal Peptide Recognition

Neural Network



Training Set

ACGAAG
AGGAAG
AGCAAG
ACGAAA
AGCAAC

EEEENN

Dersired Output

Definitions

A = [001]
C = [010]
G = [100]

E = [01]
N = [00]

Sliding Window

ACGAAG

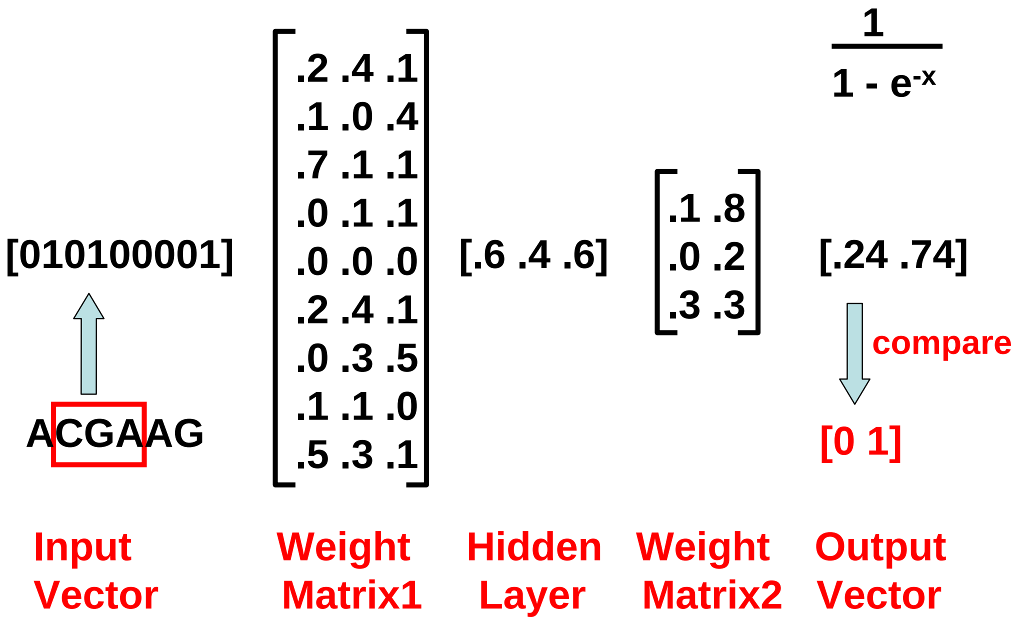
[010100001]

Input Vector

[01]

Output Vector

Neural Network Training



$$\frac{1}{1 - e^{-x}}$$

[010100001]

ACGAAG

$$\begin{bmatrix} .2 & .4 & .1 \\ .1 & .0 & .4 \\ .7 & .1 & .1 \\ .0 & .1 & .1 \\ .0 & .0 & .0 \\ .2 & .4 & .1 \\ .0 & .3 & .5 \\ .1 & .1 & .0 \\ .5 & .3 & .1 \end{bmatrix}$$

[.6 .4 .6]

$$\begin{bmatrix} .1 & .8 \\ .0 & .2 \\ .3 & .3 \end{bmatrix}$$

[.24 .74]

compare

[0 1]

Input Vector

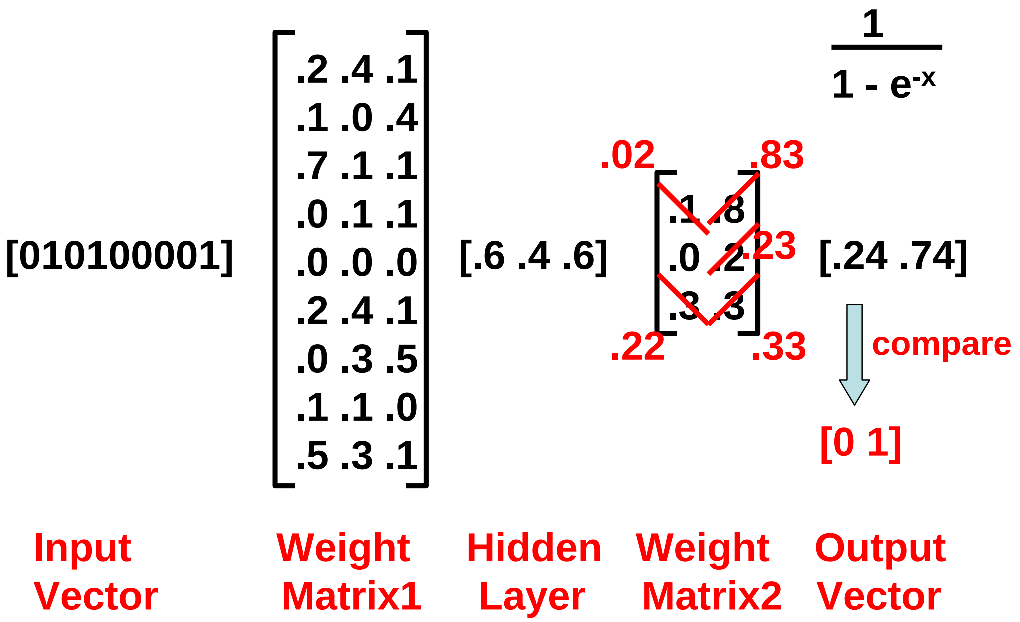
Weight Matrix1

Hidden Layer

Weight Matrix2

Output Vector

Back Propagation



$$\frac{1}{1 - e^{-x}}$$

[010100001]

$$\begin{bmatrix} .2 & .4 & .1 \\ .1 & .0 & .4 \\ .7 & .1 & .1 \\ .0 & .1 & .1 \\ .0 & .0 & .0 \\ .2 & .4 & .1 \\ .0 & .3 & .5 \\ .1 & .1 & .0 \\ .5 & .3 & .1 \end{bmatrix}$$

[.6 .4 .6]

$$\begin{bmatrix} \cancel{.1} & \cancel{.8} \\ .0 & \cancel{.2} \\ \cancel{.3} & \cancel{.3} \end{bmatrix}$$

.02 .83

.23

.22 .33

[.24 .74]

compare

[0 1]

Input Vector

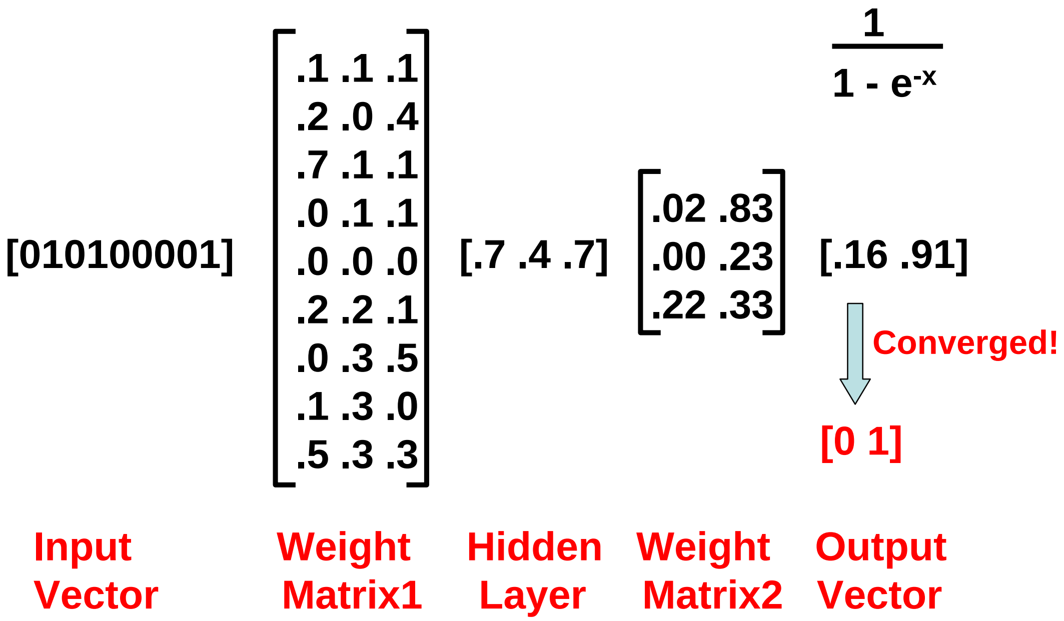
Weight Matrix1

Hidden Layer

Weight Matrix2

Output Vector

Calculate New Output



$$\frac{1}{1 - e^{-x}}$$

[010100001]

$$\begin{bmatrix} .1 & .1 & .1 \\ .2 & .0 & .4 \\ .7 & .1 & .1 \\ .0 & .1 & .1 \\ .0 & .0 & .0 \\ .2 & .2 & .1 \\ .0 & .3 & .5 \\ .1 & .3 & .0 \\ .5 & .3 & .3 \end{bmatrix}$$

[.7 .4 .7]

$$\begin{bmatrix} .02 & .83 \\ .00 & .23 \\ .22 & .33 \end{bmatrix}$$

[.16 .91]

Converged!

[0 1]

Input Vector

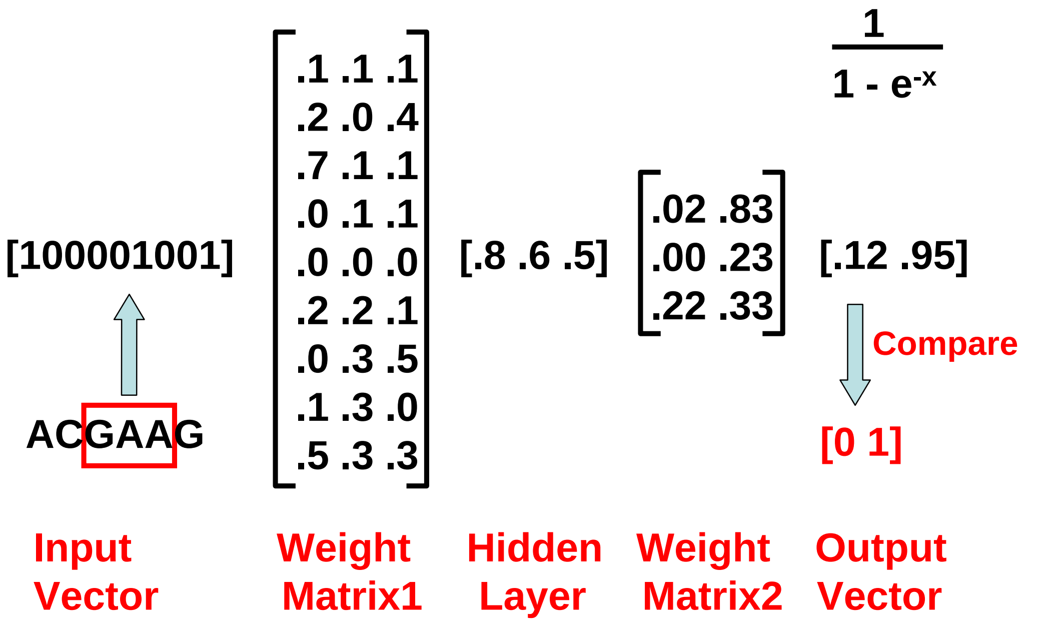
Weight Matrix1

Hidden Layer

Weight Matrix2

Output Vector

Train on Second Input Vector



$$\frac{1}{1 - e^{-x}}$$

[100001001]

ACGAAG

$$\begin{bmatrix} .1 & .1 & .1 \\ .2 & .0 & .4 \\ .7 & .1 & .1 \\ .0 & .1 & .1 \\ .0 & .0 & .0 \\ .2 & .2 & .1 \\ .0 & .3 & .5 \\ .1 & .3 & .0 \\ .5 & .3 & .3 \end{bmatrix}$$

[.8 .6 .5]

$$\begin{bmatrix} .02 & .83 \\ .00 & .23 \\ .22 & .33 \end{bmatrix}$$

[.12 .95]

Compare

[0 1]

Input Vector

Weight Matrix1

Hidden Layer

Weight Matrix2

Output Vector

Back Propagation

$$\frac{1}{1 - e^{-x}}$$

$$[010100001] \quad \begin{bmatrix} .1 & .1 & .1 \\ .2 & .0 & .4 \\ .7 & .1 & .1 \\ .0 & .1 & .1 \\ .0 & .0 & .0 \\ .2 & .2 & .1 \\ .0 & .3 & .5 \\ .1 & .3 & .0 \\ .5 & .3 & .3 \end{bmatrix} \quad [.8\ .6\ .5] \quad \begin{bmatrix} \overset{.01}{\cancel{.02}} & \overset{.84}{\cancel{.83}} \\ .00 & \cancel{.23} \end{bmatrix}\!\!\!\begin{matrix} \\ .24 \end{matrix} \!\!\!\!\!\! \begin{matrix} \\ \end{matrix} [.12\ .95]$$

$$\begin{bmatrix} \underset{.21}{\cancel{.22}} & \underset{.34}{\cancel{.33}} \end{bmatrix}$$

$\downarrow$ compare

[0 1]

Input Vector

Weight Matrix1

Hidden Layer

Weight Matrix2

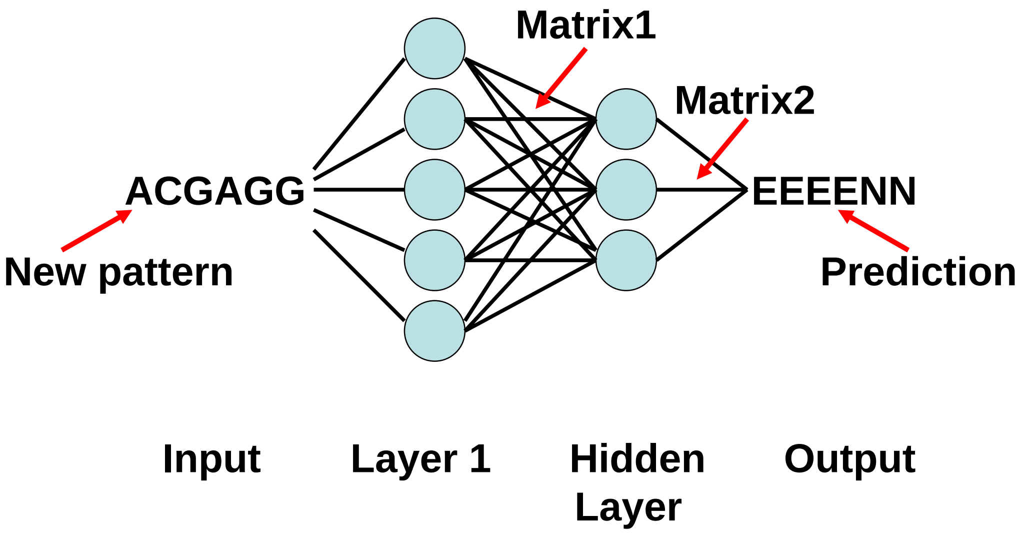
Output Vector

After Many Iterations….

$$\begin{bmatrix} .13 & .08 & .12 \\ .24 & .01 & .45 \\ .76 & .01 & .31 \\ .06 & .32 & .14 \\ .03 & .11 & .23 \\ .21 & .21 & .51 \\ .10 & .33 & .85 \\ .12 & .34 & .09 \\ .51 & .31 & .33 \end{bmatrix} \qquad \begin{bmatrix} .03 & .93 \\ .01 & .24 \\ .12 & .23 \end{bmatrix}$$

Two “Generalized” Weight Matrices

Neural Networks



Matrix1

Matrix2

ACGAGG

EEEENN

New pattern

Prediction

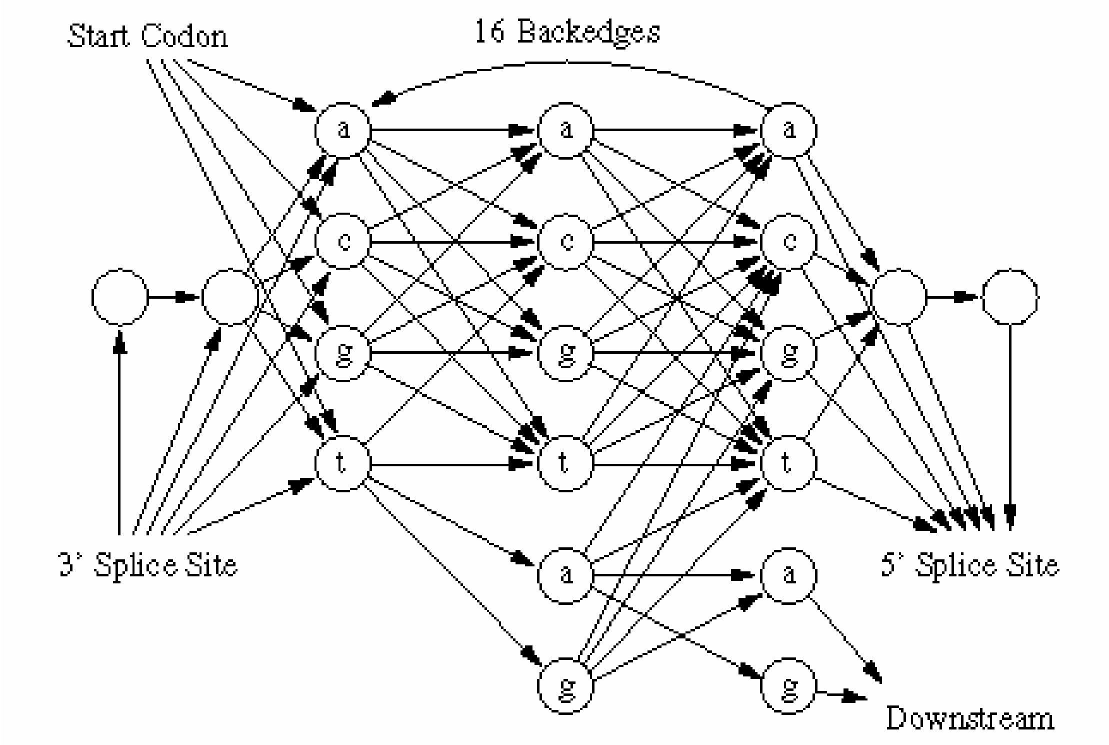
Input

Layer 1

Hidden Layer

Output

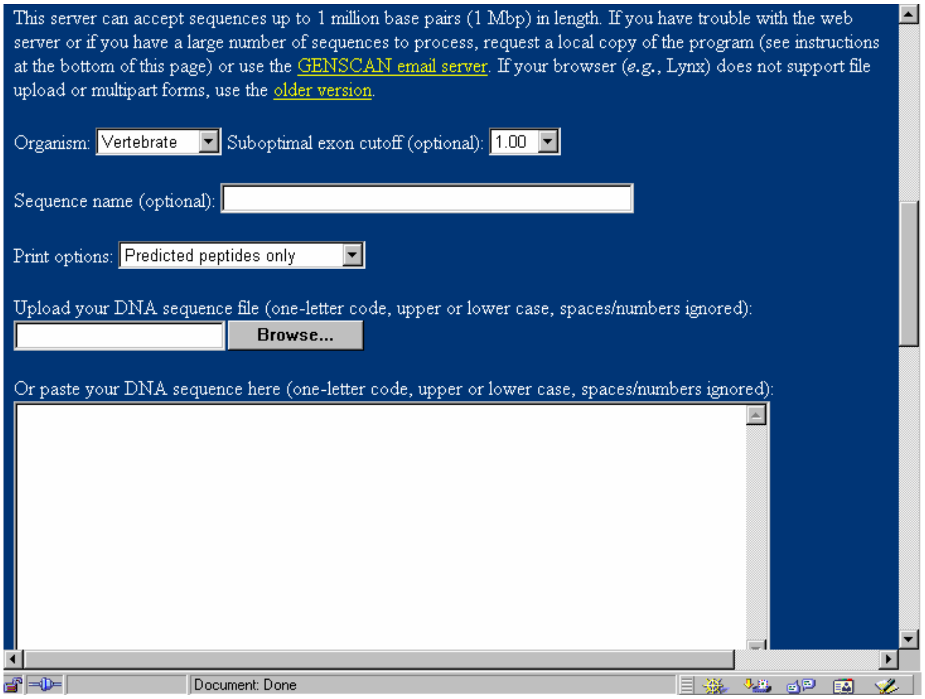
HMM for Gene Finding



Combined Methods

- Bring 2 or more methods together (usually site detection + composition)
- GRAIL (http://compbio.ornl.gov/Grail-1.3/)
- FGENEH (http://genomic.sanger.ac.uk/gf/gf.shtml)
- HMMgene (http://www.cbs.dtu.dk/services/HMMgene/)
- GENSCAN(http://genes.mit.edu/GENSCAN.html)
- Gene Parser (http://beagle.colorado.edu/~eesnyder/GeneParser.html)
- GRPL (GeneTool/BioTools)

Genscan



This server can accept sequences up to 1 million base pairs (1 Mbp) in length. If you have trouble with the web server or if you have a large number of sequences to process, request a local copy of the program (see instructions at the bottom of this page) or use the GENSCAN email server. If your browser (*e.g.*, Lynx) does not support file upload or multipart forms, use the older version.

Organism: Vertebrate Suboptimal exon cutoff (optional): 1.00

Sequence name (optional):

Print options: Predicted peptides only

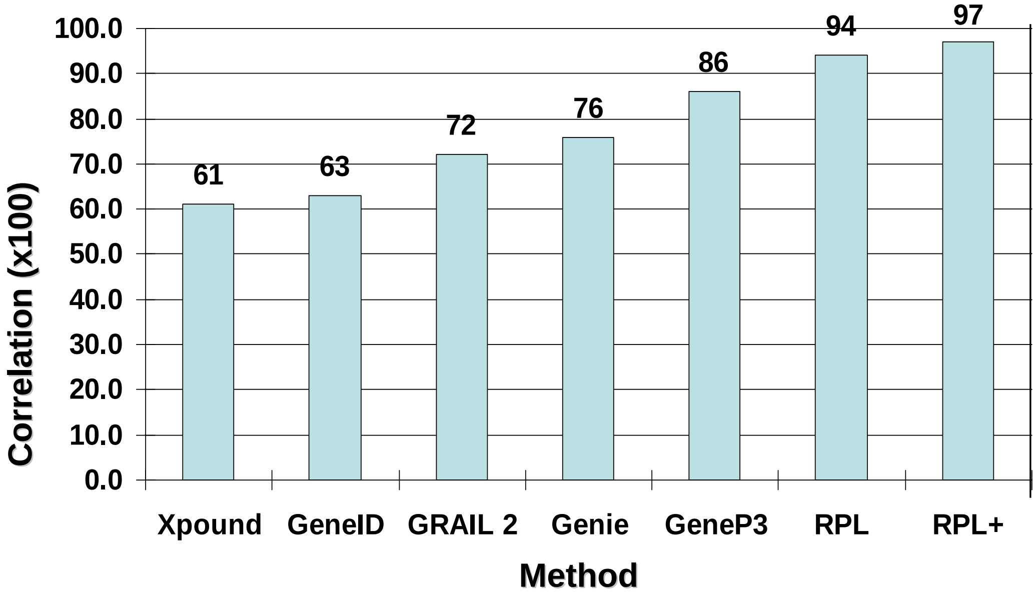
Upload your DNA sequence file (one-letter code, upper or lower case, spaces/numbers ignored):

Or paste your DNA sequence here (one-letter code, upper or lower case, spaces/numbers ignored):

How Do They Work?

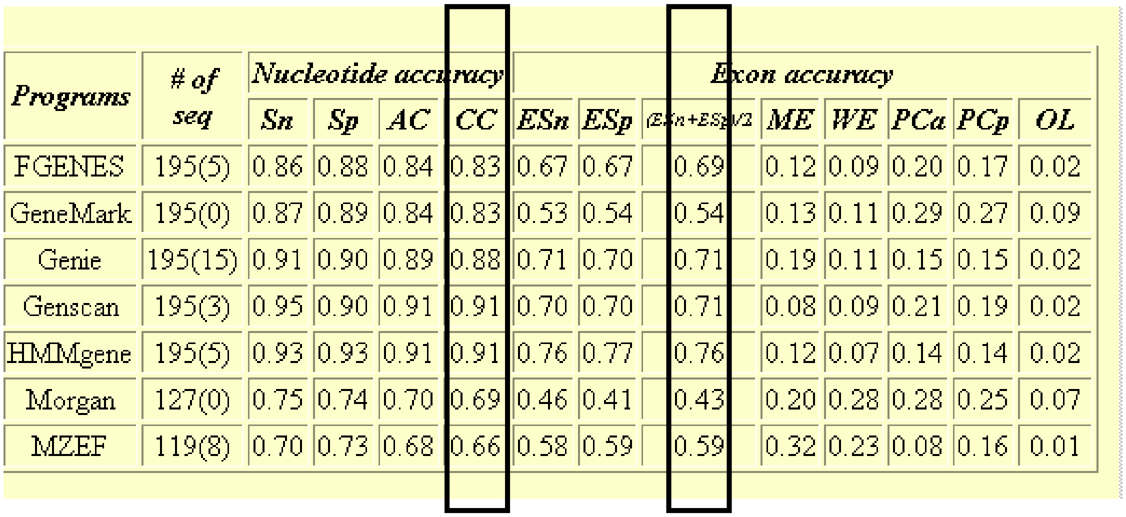
- GENSCAN
  - 5th order Hidden Markov Model
  - Hexamer composition statistics of exons vs. introns
  - Exon/intron length distributions
  - Scan of promoter and polyA signals
  - Weight matrices of 5’ splice signals and start codon region (12 bp)
  - Uses dynamic programming to optimize gene model using above data

How Well Do They Do?



Burset & Guigio test set (1996)

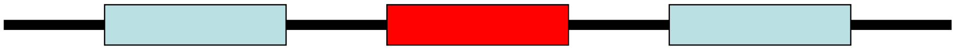
How Well Do They Do?



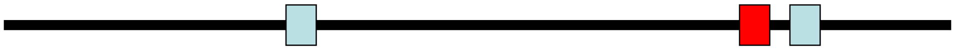
Programs	# of seq	Nucleotide accuracy				Exon accuracy							
		Sn	Sp	AC	CC	ESn	ESp	(ESn+ESp)/2	ME	WE	PCa	PCp	OL
FGENES	195(5)	0.86	0.88	0.84	0.83	0.67	0.67	0.69	0.12	0.09	0.20	0.17	0.02
GeneMark	195(0)	0.87	0.89	0.84	0.83	0.53	0.54	0.54	0.13	0.11	0.29	0.27	0.09
Genie	195(15)	0.91	0.90	0.89	0.88	0.71	0.70	0.71	0.19	0.11	0.15	0.15	0.02
Genscan	195(3)	0.95	0.90	0.91	0.91	0.70	0.70	0.71	0.08	0.09	0.21	0.19	0.02
HMMgene	195(5)	0.93	0.93	0.91	0.91	0.76	0.77	0.76	0.12	0.07	0.14	0.14	0.02
Morgan	127(0)	0.75	0.74	0.70	0.69	0.46	0.41	0.43	0.20	0.28	0.28	0.25	0.07
MZEF	119(8)	0.70	0.73	0.68	0.66	0.58	0.59	0.59	0.32	0.23	0.08	0.16	0.01

"Evaluation of gene finding programs" S. Rogic, A. K. Mackworth and B. F. F. Ouellette. Genome Research, 11: 817-832 (2001).

Easy vs. Hard Predictions

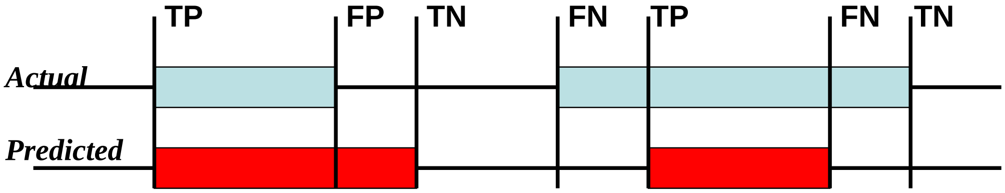


3 equally abundant states (easy)
BUT random prediction = 33% correct



Rare events, unequal distribution (hard)
BUT “biased” random prediction = 90% correct

Gene Prediction (Evaluation)



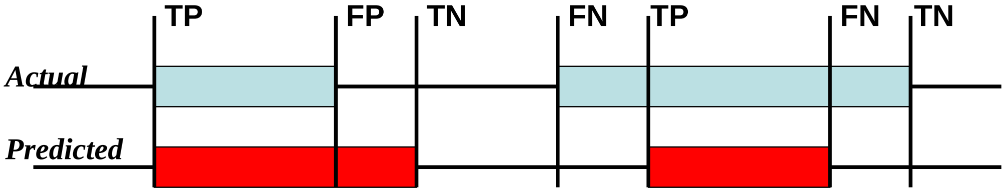
Sensitivity *Measure of the % of false negative results (sn = 0.996 means 0.4% false negatives)*

Specificity *Measure of the % of false positive results*

Precision *Measure of the % positive results*

Correlation *Combined measure of sensitivity and specificity*

Gene Prediction (Evaluation)



Sensitivity or Recall $Sn=TP/(TP + FN)$

Specificity $Sp=TN/(TN + FP)$

Precision $Pr=TP/(TP + FP)$

Correlation

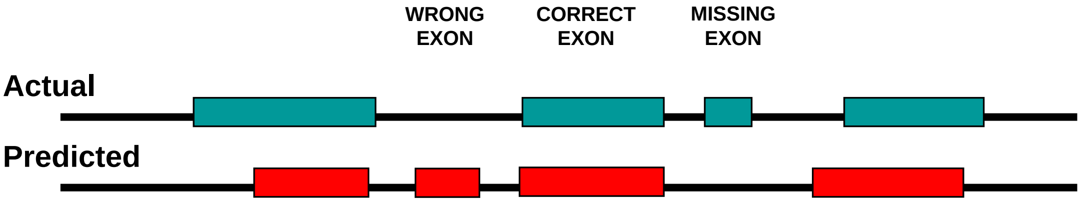
$$CC=(TP*TN-FP*FN)/[(TP+FP)(TN+FN)(TP+FN)(TN+FP)]^{0.5}$$

This is a better way of evaluating

Different Strokes for Different Folks

- Precision and specificity statistics favor conservative predictors that make no prediction when there is doubt about the correctness of a prediction, while the sensitivity (recall) statistic favors liberal predictors that make a prediction if there is a chance of success.
- Information retrieval papers report precision and recall,while bioinformaticspapers tend to report specificity and sensitivity.

Gene Prediction Accuracy at the Exon Level



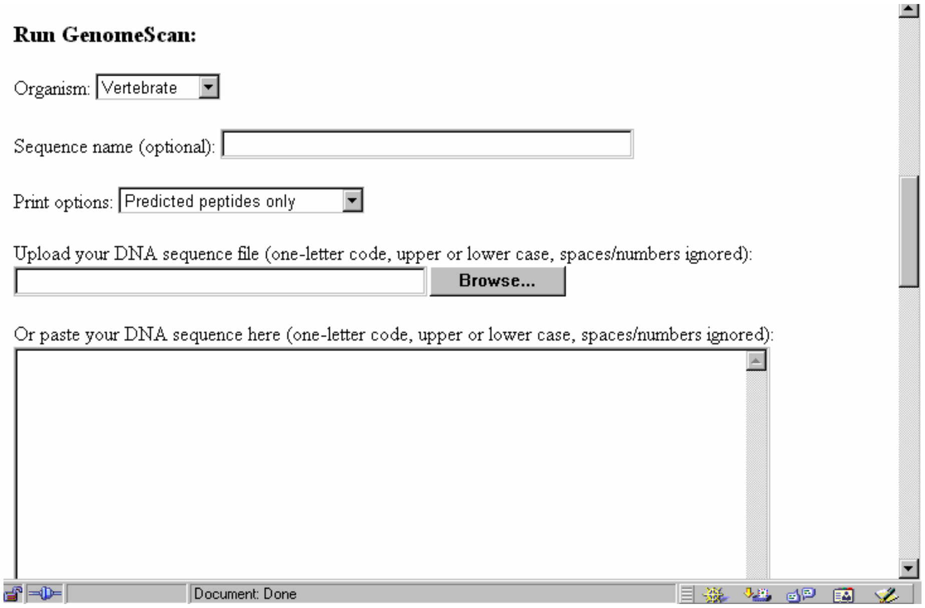
Sensitivity $S_n = \frac{\text{number of correct exons}}{\text{number of actual exons}}$

Specificity $S_p = \frac{\text{number of correct exons}}{\text{number of predicted exons}}$

Better Approaches Are Emerging...

- Programs that combine site, comparative and composition (3 in 1)
  - GenomeScan, FGENESH++, Twinscan
- Programs that use synteny between organisms
  - ROSETTA, SLAM, SGP
- Programs that combine predictions from multiple predictors
  - GeneComber, DIGIT

GenomeScan - http://genes.mit.edu/genomescan.html



Run GenomeScan:

Organism: Vertebrate

Sequence name (optional):

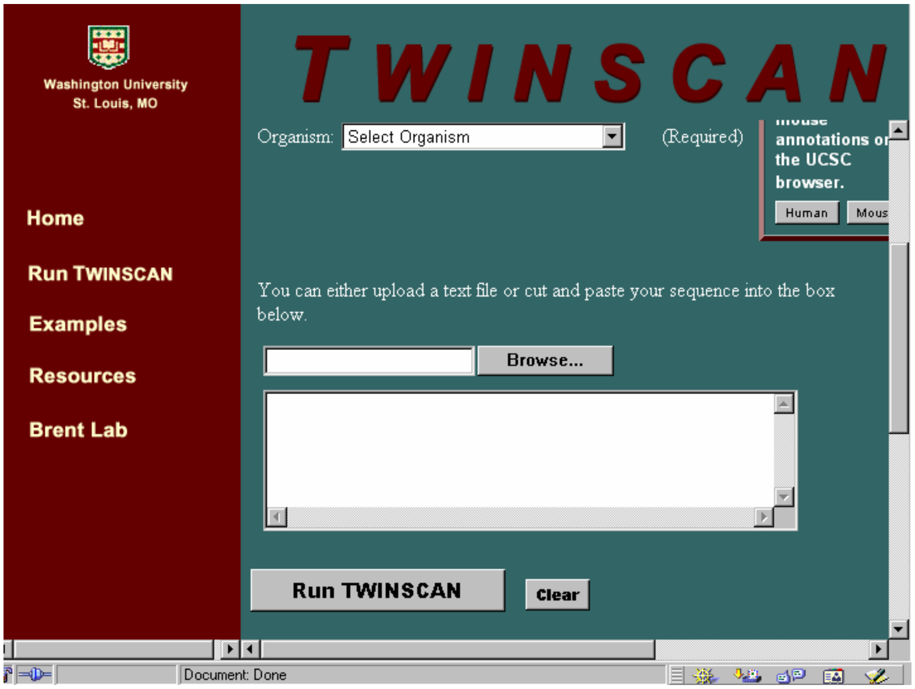
Print options: Predicted peptides only

Upload your DNA sequence file (one-letter code, upper or lower case, spaces/numbers ignored):

Or paste your DNA sequence here (one-letter code, upper or lower case, spaces/numbers ignored):

Document: Done

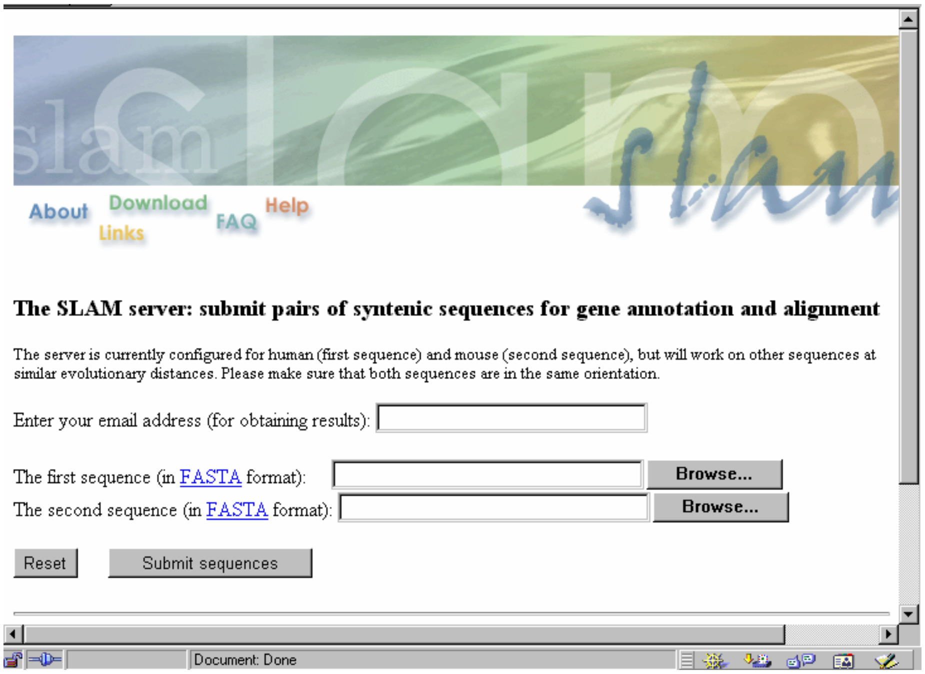
TwinScan - http://genes.cs.wustl.edu/



Organism: Select Organism (Required)

You can either upload a text file or cut and paste your sequence into the box below.

SLAM - http://baboon.math.berkeley.edu/~syntenic/slam.html



The SLAM server: submit pairs of syntenic sequences for gene annotation and alignment

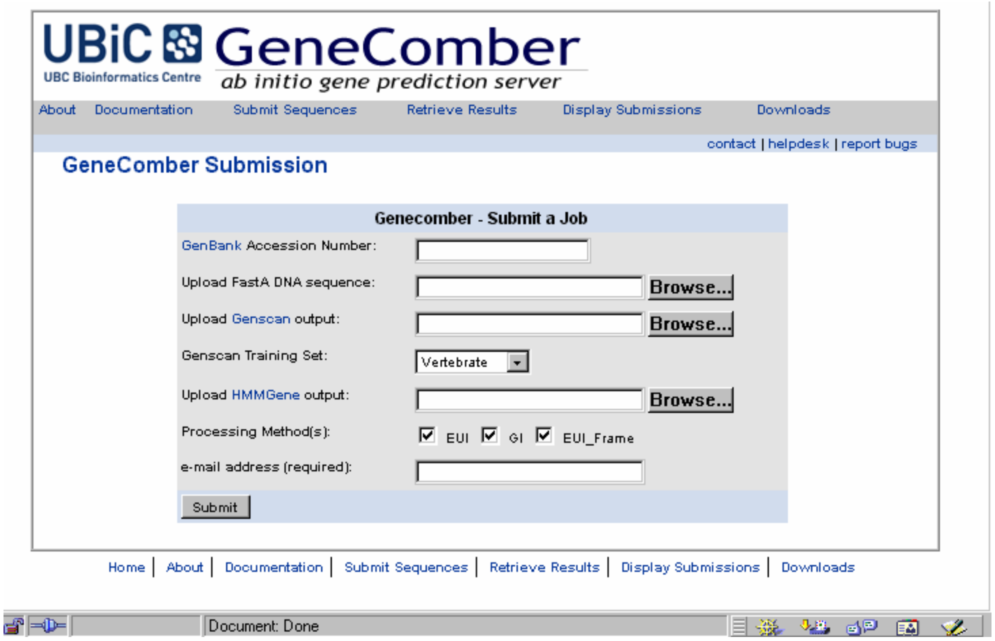
The server is currently configured for human (first sequence) and mouse (second sequence), but will work on other sequences at similar evolutionary distances. Please make sure that both sequences are in the same orientation.

Enter your email address (for obtaining results):

The first sequence (in FASTA format):

The second sequence (in FASTA format):

GeneComber - http://www.bioinformatics.ubc.ca/genecomber/submit.php


UBiC
UBC Bioinformatics Centre
GeneComber
ab initio gene prediction server
About
Documentation
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Retrieve Results
Display Submissions
Downloads
contact | helpdesk | report bugs
Home | About | Documentation | Submit Sequences | Retrieve Results | Display Submissions | Downloads
Document: Done

GeneComber Submission

Genecomber - Submit a Job

GenBank Accession Number:

Upload FastA DNA sequence: Browse...

Upload Genscan output: Browse...

Genscan Training Set: Vertebrate

Upload HMMGene output: Browse...

Processing Method(s): ☑ EUI ☑ GI ☑ EUI_Frame

e-mail address (required):

Submit

Outstanding Issues

- Most Gene finders don’t handle UTRs (untranslated regions)
- ~40% of human genes have non-coding 1st exons (UTRs)
- Most gene finders don’t’ handle alternative splicing
- Most gene finders don’t handle overlapping or nested genes
- Most can’t find non-protein genes (tRNAs)

Bottom Line...

- Gene finding in eukaryotes is not yet a “solved” problem
- Accuracy of the best methods approaches 80% at the exon level (90% at the nucleotide level) in coding-rich regions (much lower for whole genomes)
- Gene predictions should always be verified by other means (cDNA sequencing, BLAST search, Mass spec.)