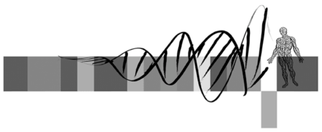

Bioinformatics for Biologists

Sequence Analysis: Part II. Pattern Searching and Gene Finding

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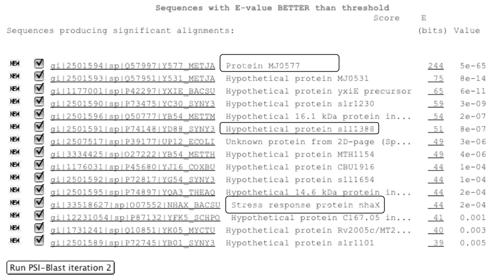
Topics to Cover

- Pattern searching
  - – PSI-BLAST
  - – PHI-BLAST
  - – Finding patterns
- Gene finding

PSI-BLAST

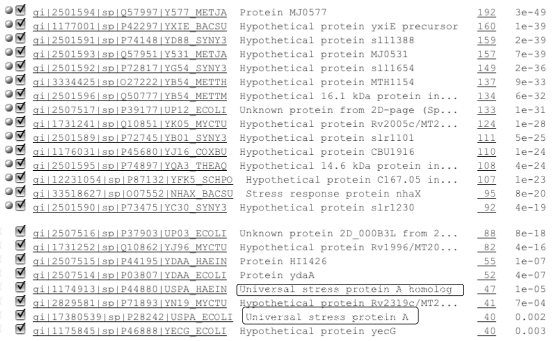
- **P**osition **S**pecific **I**terative BLAST uses a profile (or position specific scoring matrix, PSSM) that is constructed (automatically) from a multiple alignment of the highest scoring hits in an initial BLAST search.
- The PSSM is generated by calculating position-specific scores for each position in the alignment. Highly conserved positions receive high scores and weakly conserved positions receive scores near zero.
- The profile is used to perform a second (etc.) BLAST search and the results of each "iteration" is used to refine the profile. This iterative searching strategy results in increased sensitivity.

Start with a BLASTP search



```
                    Sequences with E-value BETTER than threshold
                                                                    Score    E
Sequences producing significant alignments:                         (bits) Value

NEW ☑ gi|2501594|sp|Q57997|Y577_METJA  Protein MJ0577                    244  5e-65
NEW ☑ gi|2501593|sp|Q57951|Y531_METJA  Hypothetical protein MJ0531        75  8e-14
NEW ☑ gi|1177001|sp|P42297|YXIE_BACSU  Hypothetical protein yxiE precursor 65  6e-11
NEW ☑ gi|2501590|sp|P73475|YC30_SYNY3  Hypothetical protein slr1230       59  3e-09
NEW ☑ gi|2501596|sp|Q50777|YB54_METTM  Hypothetical 16.1 kDa protein in... 54  2e-07
NEW ☑ gi|2501591|sp|P74148|YD88_SYNY3  Hypothetical protein sll1388       51  8e-07
NEW ☑ gi|2507517|sp|P39177|UP12_ECOLI  Unknown protein from 2D-page (Sp... 49  3e-06
NEW ☑ gi|3334425|sp|O27222|YB54_METTH  Hypothetical protein MTH1154       49  4e-06
NEW ☑ gi|1176031|sp|P45680|YJ16_COXBU  Hypothetical protein CBU1916       44  1e-04
NEW ☑ gi|2501592|sp|P72817|YG54_SYNY3  Hypothetical protein sll1654       44  1e-04
NEW ☑ gi|2501595|sp|P74897|YQA3_THEAQ  Hypothetical 14.6 kDa protein in... 44  2e-04
NEW ☑ gi|33518627|sp|O07552|NHAX_BACSU  Stress response protein nhaX     44  2e-04
NEW ☑ gi|12231054|sp|P87132|YFK5_SCHPO  Hypothetical protein C167.05 in... 41  0.001
NEW ☑ gi|1731241|sp|Q10851|YK05_MYCTU  Hypothetical protein Rv2005c/MT2... 40  0.003
NEW ☑ gi|2501589|sp|P72745|YB01_SYNY3  Hypothetical protein slr1101       39  0.005
```

PSI-BLAST - Iteration 1



```
● ☑ gi|2501594|sp|Q57997|Y577_METJA  Protein MJ0577                      192  3e-49
● ☑ gi|1177001|sp|P42297|YXIE_BACSU  Hypothetical protein yxiE precursor  160  1e-39
● ☑ gi|2501591|sp|P74148|YD88_SYNY3  Hypothetical protein sll1388         159  2e-39
● ☑ gi|2501593|sp|Q57951|Y531_METJA  Hypothetical protein MJ0531          157  7e-39
● ☑ gi|2501592|sp|P72817|YG54_SYNY3  Hypothetical protein sll1654         149  2e-36
● ☑ gi|3334425|sp|O27222|YB54_METTH  Hypothetical protein MTH1154         137  9e-33
● ☑ gi|2501596|sp|Q50777|YB54_METTM  Hypothetical 16.1 kDa protein in...  134  6e-32
● ☑ gi|2507517|sp|P39177|UP12_ECOLI  Unknown protein from 2D-page (Sp...  133  1e-31
● ☑ gi|1731241|sp|Q10851|YK05_MYCTU  Hypothetical protein Rv2005c/MT2...  124  1e-28
● ☑ gi|2501589|sp|P72745|YB01_SYNY3  Hypothetical protein slr1101         111  5e-25
● ☑ gi|1176031|sp|P45680|YJ16_COXBU  Hypothetical protein CBU1916         110  1e-24
● ☑ gi|2501595|sp|P74897|YQA3_THEAQ  Hypothetical 14.6 kDa protein in...  108  4e-24
● ☑ gi|12231054|sp|P87132|YFK5_SCHPO  Hypothetical protein C167.05 in...  107  1e-23
● ☑ gi|33518627|sp|O07552|NHAX_BACSU  Stress response protein nhaX         95  8e-20
● ☑ gi|2501590|sp|P73475|YC30_SYNY3  Hypothetical protein slr1230          92  4e-19

NEW ☑ gi|2507516|sp|P37903|UP03_ECOLI  Unknown protein 2D_000B3L from 2...  88  8e-18
NEW ☑ gi|1731252|sp|Q10862|YJ96_MYCTU  Hypothetical protein Rv1996/MT20...  82  4e-16
NEW ☑ gi|2507515|sp|P44195|YDAA_HAEIN  Protein HI1426                       55  1e-07
NEW ☑ gi|2507514|sp|P03807|YDAA_ECOLI  Protein ydaA                         52  4e-07
NEW ☑ gi|1174913|sp|P44880|USPA_HAEIN  Universal stress protein A homolog   47  1e-05
NEW ☑ gi|2829581|sp|P71893|YN19_MYCTU  Hypothetical protein Rv2319c/MT2...  41  7e-04
NEW ☑ gi|17380539|sp|P28242|USPA_ECOLI  Universal stress protein A          40  0.002
NEW ☑ gi|1175845|sp|P46888|YECG_ECOLI  Hypothetical protein yecG            40  0.003
```

Amino acids

PSSM from PSI-BLAST

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
1	0	2	3	2	4	1	1	4	3	0	3	3	7	3	3	2	1	0	1	2
2	6	0	3	3	5	4	0	3	2	5	0	1	2	2	4	1	3	2	4	2
3	4	3	0	3	3	1	3	2	4	2	3	2	5	0	1	2	1	0	5	7
4	3	2	3	2	4	9	3	3	5	4	0	3	2	5	1	2	2	4	1	2
5	0	1	2	2	4	1	6	3	3	1	3	2	0	4	8	3	1	0	3	0
6	4	3	2 …																	
•	…																			
•	…																			
N																				

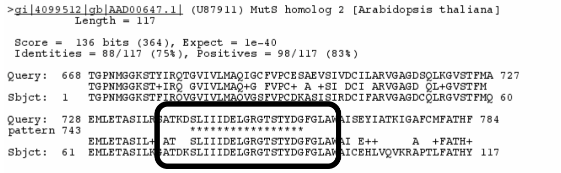
POSITIONS

Pattern Hit Initiated (PHI)-BLAST

```
>HUMAN MSH2

MAVQPKETLQLESAAEVGFVRFFQGMPEKPTTTVRLFDRGDFYTAHGEDALLAAREVFKTQGVIKYMGPA
GAKNLQSVVLSKMNFESFVKDLLLVRQYRVEVYKNRAGNKASKENDWYLAYKASPGNLSQFEDILFGNND
MSASIGVVGVKMSAVDGQRQVGVGYVDSIQRKLGLCEFPDNDQFSNLEALLIQIGPKECVLPGGETAGDM
GKLRQIIQRGGILITERKKADFSTKDIYQDLNRLLKGKKGEQMNSAVLPEMENQVAVSSLSAVIKFLELL
SDDSNFGQFELTTFDFSQYMKLDIAAVRALNLFQGSVEDTTGSQSLAALLNKCKTPQGQRLVNQWIKQPL
MDKNRIEERLNLVEAFVEDAELRQTLQEDLLRRFPDLNRLAKKFQRQAANLQDCYRLYQGINQLPNVIQA
LEKHEGKHQKLLLAVFVTPLTDLRSDFSKFQEMIETTLDMDQVENHEFLVKPSFDPNLSELREIMNDLEK
KMQSTLISAARDLGLDPGKQIKLDSSAQFGYYFRVTCKEEKVLRNNKNFSTVDIQKNGVKFTNSKLTSLN
EEYTKNKTEYEEAQDAIVKEIVNISSGYVEPMQTLNDVLAQLDAVVSFAHVSNGAPVPYVRPAILEKGQG
RIILKASRHACVEVQDEIAFIPNDVYFEKDKQMFHIITGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESA
EVSIVDCILARVGAGDSQLKGVSTFMAEMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISEYI
ATKIGAFCMFATHFHELTALANQIPTVNNLHVTALTTEETLTMLYQVKKGVCDQSFGIHVAELANFPKHV
               FQYIGESQGYDIMEPAAKKCYLEREQGEKIIQEFLSKVKQMPFTEMSEENITIKLKQ
               NEIISRIKVTT
```

DNA mismatch repair proteins mutS family signature

PHI-BLAST



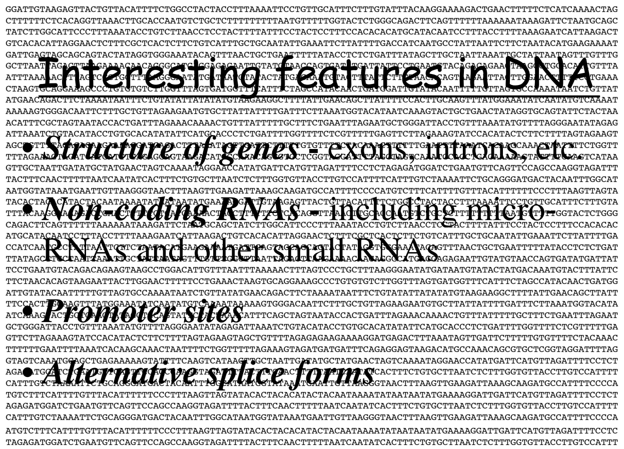
```
>gi|4099512|gb|AAD00647.1| (U87911) MutS homolog 2 [Arabidopsis thaliana]
          Length = 117

 Score =  136 bits (364), Expect = 1e-40
 Identities = 88/117 (75%), Positives = 98/117 (83%)

Query:  668 TGPNMGGKSTYIRQTGVIVLMAQIGCFVPCESAEVSIVDCILARVGAGDSQLKGVSTFMA 727
            TGPNMGGKST+IRQ GVIVLMAQ+G FVPC+ A +SI DCI ARVGAGD QL+GVSTFM
Sbjct:  1   TGPNMGGKSTFIRQVGVIVLMAQVGSFVPCDKASISIRDCIFARVGAGDCQLRGVSTFMQ 60

Query:  728 EMLETASILRSATKDSLIIIDELGRGTSTYDGFGLAWAISEYIATKIGAFCMFATHF 784
pattern 743                ****************
            EMLETASIL+ AT  SLIIIDELGRGTSTYDGFGLAWAI E++     A  +FATH+
Sbjct:  61  EMLETASILKGATDKSLIIIDELGRGTSTYDGFGLAWAICEHLVQVKRAPTLFATHY 117
```

Pattern Searching

RRRRYYYY or R(4)Y(4)	4 purines followed by 4 pyrimidines
TATAA	Exact pattern: TATAA
[DE](2)HS{P}X(2)PX(2,4)C	[ ] Acceptable { } Not acceptable X(2) 2 of anything in a row X(2,4) from 2 to four of of anything
p1=6...8 GAGA ~p1	hairpin with GAGA as the loop
p1=6...6 3...8 p1	exact 6 character repeat separated by up to 8
p1=6...6 3..8 p1[1,1,1]	allow one mismatch, deletion and insertion

Pattern Searching Programs

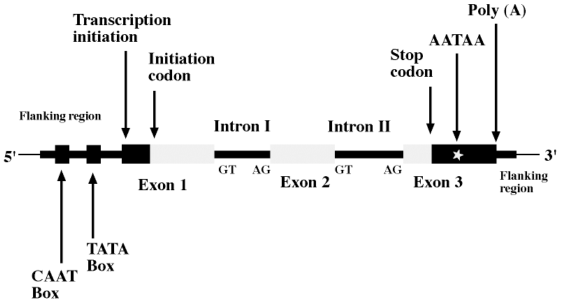
Patscan	scan_for_matches patfile < inputfile
fuzznuc, fuzzprot, fuzztrans, dreg	EMBOSS programs; web and Unix



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



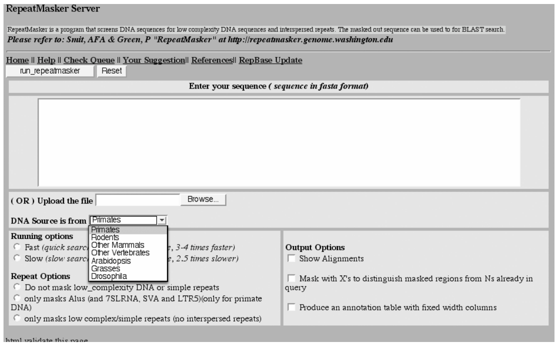
Types of Signals to Detect

- 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

RepeatMasker



RepeatMasker Server

RepeatMasker is a program that screens DNA sequences for low complexity DNA sequences and interspersed repeats. The masked out sequence can be used to for BLAST search.

Please refer to: Smit, AFA & Green, P "RepeatMasker" at http://repeatmasker.genome.washington.edu

Home || Help || Check Queue || Your Suggestion|| References|| RepBase Update
run_repeatmasker
Reset

Enter your sequence *(sequence in fasta format)*

(OR) Upload the file

DNA Source is from Primates

Running options

○ Fast *(quick searc* *e, 3-4 times faster)*
○ Slow *(slow searc* *e, 2.5 times slower)*

Repeat Options

○ Do not mask low_complexity DNA or simple repeats
○ only masks Alus (and 7SLRNA, SVA and LTR5)(only for primate DNA)
○ only masks low complex/simple repeats (no interspersed repeats)

Output Options

□ Show Alignments
□ Mask with X's to distinguish masked regions from Ns already in query
□ Produce an annotation table with fixed width columns

RepeatMasker

Summary:

Total length: 8750 bp

GC level: 35.61%

Bases masked: 6803 bp (77.75%)

```
==================================================
               number of      length   percentage
               elements*    occupied  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
  <u>ACCTCG</u> <u>TACTCG</u> <u>GCCCTC</u>
  Thr Ser Tyr Ser Ala Leu

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

Gene Finding Programs

- FGENESH - Softberry
- GeneID - Barcelona
- Genscan - Stanford and MIT
- GenomeScan - MIT
- MZEF/First exon - Cold Spring Harbor
- Twinscan - WU
- Ecgene - Korea (EST clustering)
- SGP - Barcelona (human-mouse homology)

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

Definitions

- ***Sensitivity:*** the proportion of true sites(e.g., exons or donor splice sites) that are correctly predicted = TP/(TP + FN)
- ***Predictive value of positive results ("Specificity" in gene finding literature):*** the proportion of predicted sites that are correct = TP/(TP + FP)
- ***Specificity:*** the proportion of non-sites that are predicted to be non-sites = TN/(TN +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

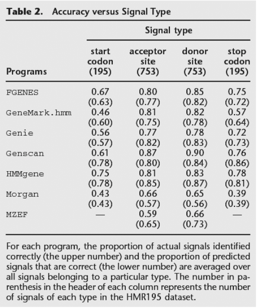
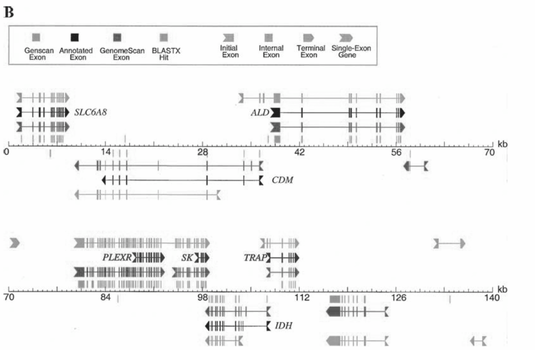


Table 2. Accuracy versus Signal Type

	Signal type			
Programs	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)	—

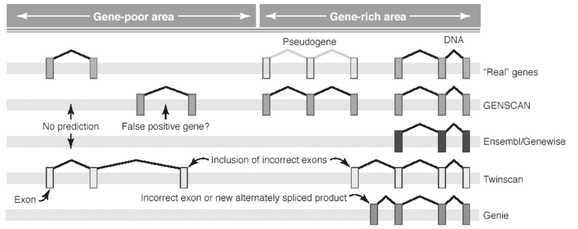
For each program, the proportion of actual signals identified correctly (the upper number) and the proportion of predicted signals that are correct (the lower number) are averaged over all signals belonging to a particular type. The number in parenthesis in the header of each column represents the number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

GenomeScan



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