NAME

SplicePredictor − Splice Site Prediction with Bayesian Models

SYNOPSIS

SplicePredictor [**−s** *species*] **−c** *cutoff*] [**−t** *pval*] [**−T** *sval*] [**−oO**] [**−n** *topN*] [**−u**] [**−U**] [**−p** *pstyle*] [**−w** *[nsites sscwl]*] [**−x**] [**−eE** *estdbn*] [**−i** *prmfile*] [**−qQ** *qpfname*] [**−I** *matname*] [**−a** *from*] [**−b** *to*] [**−rR**] [**−lL** *libname*] [**−g** *gbfname(s)*]

DESCRIPTION

The basic version of *SplicePredictor* implements Bayesian models for splice site prediction trained as described in References 1 and 2. The predictions are implicitely based on the three variables of (i) degree of matching to the splice site consensus, (ii) local compositional contrast, and (iii) assessment of 3-base periodicity in coding regions. The models assign a *P-value* between 0 and 1 to each potential splice site such that true sites mostly score high and non-sites mostly score low. The *P-value*s represent intrinsic splice site quality (see also Reference 3). Refinements of the basic model include the context-dependent scores *rho* and *gamma* (Reference 4). The *rho-value* of a given site is calculated as a weighted product of its P-value times the P-value of its best potential intron-forming complementary splice site; 0 < *rho* < 1. The *gamma-value* of a site reflects how well this site fits in the locally predicted splicing pattern. If the given site is in a context that suggests preferred usage of nearby sites as splicing partners to the exclusion of the given site, its *gamma-value* will be zero. Otherwise it will be a positive value less or equal to 2; high values of *gamma* would strongly suggest actual usage of the site.

To quickly assess the overall quality of a site we implemented a * grading system: the values of *P*, *rho*, and *gamma* are labeled 5*, 4*, 3*, or 2* if they match or exceed the threshold values for 90%, 80%, 65%, and 50% prediction specificity on the training set, 1* otherwise. The sum of the *-*values* (attaining values between 3* and 15*) serves as a simple combined measure. For example, sites scoring 14* or 15* are highly reliable (estimated specificity > 90%).

Minimal input to the program consists of a genomic sequence for which potential splice sites are to be listed. Optionally, the user may also supply cDNA/ESTs or "target proteins" which are known or suspected to significantly match the genomic sequence or its translation into encoded amino acids chains. If supplied, the algorithm will return optimal *spliced alignments* which "thread" the targets into the genomic DNA by scoring for splice sites and sequence similarity in potential exons while allowing for introns as long gaps in the alignment (References 5 and 6).

The **SplicePredictor** program was developed in the group of Prof. V. Brendel and is freely available under the GNU General Public Licence at http://brendelgroup.org/bioinformatics2go/SplicePredictor.php/. Correspondence relating to **SplicePredictor** should be addressed to

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OPTIONS

−s *species*
: Set *species* to select the most appropriate splice site models. This parameter must be specified. Options: "human", "mouse", "rat", "chicken", "Drosophila", "Daphnia", "nematode", "yeast", "Aspergillus", "Arabidopsis", "maize", "rice", "Medicago", "generic".

−c *cutoff*
: set prediction threshold level; only sites with critical value c = 2 ln BF at least *cutoff* will be printed

−t *pval* set prediction threshold to *pval* [overrides -c option]

−T *sval* set prediction threshold to *sval* [overrides -c option]

−oO -o: order sites by P-value [default: by position] -O: order sites by *-value [default: by position]

−n *topN*
: display top N splice sites

−u disable local pruning of non-optimal sites

−U score also non-canonical splice site dinucleotides

−p *pstyle*
: 1 (terse=WWW); 2 (default); 3 (very terse=EXDOMINO); 4 (verbose); 5 (spreadsheet)

−w [*nsites sscwl*]
report splice site clusters (>= *nsites* in <= *sscwl* bases; default: 4/1500, appropriate for -T 14 option)

−x [*from to*]
LaTex graphical output in *.tex file(s)

−eE *estdbn*
Read EST sequence data from library file *estdbn*; -e: align + strand only, -E: align + and - strands.

−i *prmfile*
Read parameters for EST matching from file *prmfile*.

−qQ *qpfname*
Read target protein sequence data from library (FASTA-format) file *qpfname*.

−I *matname*
Read amino acid substitution scoring matrix from file *matname*.

−a *from*
Analyze genomic sequence from position *from* [default: 1].

−b *to* Analyze genomic sequence up to position *to* [default: end of sequence].

−r Analyze reverse strand.

−R Analyze both strands.

−lL *libname*
Read (multiple) sequence data from library file *libfname* (FASTA-format).

−g *gbfname(s)*
Read nucleic acid sequence data from GenBank file(s) *gbfname(s)*. If specified, the -g option must be last.

USAGE

Input file format

Genomic DNA input: Sequences should be in the one-letter-code ({a,b,c,d,g,h,i,k,m,n,q,r,s,t,u,v,w,y}), upper or lower case; all other characters are ignored during input. Multiple sequence input is accepted in *library (FASTA) file format* or in *GenBank format*.

Library (FASTA) file format refers to raw sequence data separated by identifier lines of the form starting with ">" followed by the sequence name. For options **-e**, **-E**, **-q**, and **-l**, the name of the sequence is taken to be the first string on the ">" line delimited by space, tab, |, or : starting from position 5. For example, ">gi|idnumber|something-else" is given the name "idnumber". For options **-Q** and **-L**, the name of the sequence is taken to be the first string on the ">" line delimited by space, tab, |, or : starting from position 2. In the above example, the name would be "gi". Typically, this option is appropriate for sequences supplied by the user in the format ">my-sequence-name comments".

Examples (**-e**, **-E**, **-q**, and **-l** options):

```
>gi|sequence1 - upper case
ACGATTGGATCAAAATCCATGAAAGAGGGGAATCTATAGGCGGAATTGAG
CGCCAGCGACTGGCTGCCTTGGCGGGGGAGGCCTTGGCGGA

>SQ;sequence2 - upper case with numbering
       1  ACGATTGGAT CAAAATCCAT GAAAGAGGGG AATCTATAGG CGGAATTGAG
```

```
       51  CGCCAGCGAC TGGCTGCCTT GGCGGGGGAG GCCTTGGCGG A

>vb:sequence3 - lower case
acgattggatcaaaatccatgaaagaggggaatctataggcggaattgagcgccagcgac
tggctgccttggcgggggaggccttggcgga

>vb:sequence4 - mixed format
       1  ACGATTGGAT CAAAATCCAT GAAAGAGGGG AATCTATAGG GGGGGGATCT
cgccagcgac
        tggctgcct       tggcgggggg       AGGCCTTGGCGGA
```

GenBank format refers to raw sequence data with possible annotations as in standard GenBank files. Minimal requirements are the LOCUS and ORIGIN lines. Multiple sequences must be separated by // lines.

EST sequence input: EST sequences for spliced alignment may be supplied as a sequence file in library format with the *-eE estdbn* options. Spliced alignment will only be performed for genomic DNA sequences of lengths not exceeding the parameter MAXGLGTH (default: 13000).

Query protein input: Query protein sequences for spliced alignment may be supplied with the *-qQ qpfname* option, where *qpfname* is a sequence file in library format. Spliced alignment will only be performed for genomic DNA sequences of lengths not exceeding the parameter MAXGLGTH (default: 13000).

Parameters

There always is a trade-off between *sensitivity* ("How many true sites will be correctly predicted?") versus *specificity* ("How large is the number of presumably false positive predictions?"). For *SplicePredictor*, sensitivity and specificity are controlled by the critical value $c = 2 \ln BF$, where *BF* is the Bayes Factor (ratio of posterior to prior oddds that a given site is a true splice site). Higher values of *c* increase specificity but decrease sensitivity (Reference 1).

Output format

Output is directed to standard output.

Potential splice sites (example):

t	q	loc	sequence	P	c	rho	gamma	*	P*R*G*	parse
.	.....									
D	---->	35713	ccgGTttgt	0.994	10.73	0.277	1.980	13	(5 3 5)	IADADIA-D-AEEDADA
A	<-----	35819	ttattaattgcgtAGgt	0.986	9.04	0.487	1.963	14	(4 5 5)	ADADIAD-A-EEDADAD
D	-->	35859	ctgGTtctg	0.793	3.26	0.000	0.000	5	(3 1 1)	DADIADA-E-EDADADA
D	-->	35890	tatGTgatt	0.788	3.20	0.000	0.000	5	(3 1 1)	ADIADAE-E-DADADAE
D	--->	36012	aagGTacga	0.978	8.13	0.268	0.185	10	(5 3 2)	DIADAEE-D-ADADAED
A	<-----	36100	tcgtgttcattgcAGat	0.996	11.54	0.497	1.973	15	(5 5 5)	IADAEED-A-DADAEDA
D	----->	36206	acgGTaatg	0.995	11.24	0.985	1.985	15	(5 5 5)	ADAEEDA-D-ADAEDAD
A	<-----	36296	ataatttttctgcAGtc	0.990	9.67	0.985	1.985	14	(4 5 5)	DAEEDAD-A-DAEDAED
D	----->	36432	cagGTatgg	0.997	12.20	0.335	1.987	14	(5 4 5)	AEEDADA-D-AEDAEDA
A	<---	36520	acattgcgataacAGgc	1.000	17.83	0.336	0.015	10	(5 3 2)	EEDADAD-A-EDAEDIA
A	<--	36721	ttcgaatctgatcAGgt	0.985	8.97	0.000	0.000	6	(4 1 1)	EDADADA-E-DAEDIAD

```
D ----->  36722           cagGTgagt   0.955   6.68   0.939  1.939  15 (5 5 5)  DADADAE-D-AEDIADA
A <-----  36815 ggatgaatgaaacAGga     0.984   8.78   0.488  1.883  14 (4 5 5)  ADADAED-A-EDIADAE
  .......
```

Column *t*: type (D, donor, or A, acceptor)
Column *q*: quality. The length of the arrow indicates the site quality measured by the *-value:

```
-----  = *value 14-15 = highly likely (estimated specificity   >90%)
----   = *value 11-13 =    likely     (estimated specificity 60-70%)
---    = *value  8-10 =    possible   (estimated specificity 35-45%)
--     = *value  5- 7 =    uncertain  (estimated specificity 10-20%)
-      = *value  3- 4 =    doubtful   (estimated specificity   < 5%)
```

```
The arrow head points into the predicted intron.
```

Column *loc*: site location (position of first or last base of potential intron for D or A, respectively)
Column *sequence*: site sequence
Column *P*: P-value
Column *c*: cutoff c = 2 ln (BF)
Column *rho*: rho-value
Column *gamma*: gamma-value
Column *: *-value
Column *P*R*G**: individual *-values for P, rho, and gamma
Column *parse*: highest scoring assignment of the given site and the seven adjacent sites upstream and downstream as either A (acceptor), D (donor), E (exon), or I (intron)
Note: Spliced alignment with ESTs confirms introns 35713-35819, 36012-36100, 36206-36296, 36342-36520, and 36722-36815 (see file out.gbA.orig in the GeneSeqer/SplicePredictor distribution data directory).

Spliced alignment: For each significantly matching EST, the predicted gene structure based on an optimal spliced alignment is displayed. The upper line gives the genomic DNA and the lower line gives the EST sequence. Identities are indicated by vertical bars in the center line. Introns are indicated by dots, gaps in the exons by '_'. For protein spliced alignments, the alignment gives the genomic DNA sequence, its inferred protein translation (one-letter-code), and the matching parts of the target protein sequence. Identical residues are linked by "|", positively scoring substitutions by "+", and zero scoring substitutions by "." according to the amino acid substitution scoring matrix used in the alignment. Coordinates for the predicted exons and introns are given in the list preceding the alignment. Exons are assigned a normalized similarity score (1.000 represents 100% identity). For introns, the list gives adjusted P-values of the donor and acceptor sites (2 * (P - 0.5) for P > 0.5) as well as a similarity score (s) based on the sequence similarity in the adjacent 50 bases of exon.

Special lines:
MATCH gDNAx cDNAy scr lgth cvrg y

where gDNA = name of genomic DNA sequence; x = + (forward strand) or - (reverse strand); cDNA = name of cDNA sequence; y = + (forward strand) or - (reverse strand); scr = alignment score; lgth = cumulative length of scored exons; cvrg = coverage of genomic DNA segment (y = G) or cDNA (y = C) or target protein (y = P), whichever is highest

PGS_gDNAx_cDNAy (a b,c d, ...)
or
PGS_gDNAx_qp (a b,c d, ...)

where gDNA = name of genomic DNA sequence; x = + (forward strand) or - (reverse strand); cDNA = name of cDNA sequence; y = + (forward strand) or - (reverse strand); qp = name of target protein; a, b, c, d, ... = exon coordinates.

The MATCH and PGS lines are useful for summarizing the search results for an application involving multiple genomic DNA sequences and multiple ESTs or target proteins (use a combination of 'egrep' and 'sort'). PGS = Predicted Gene Structure (GenBank CDS-styled exon coordinates).

NOTES

The **SplicePredictor.c** source code includes also the older logitlinear models for maize and *Arabidopsis thaliana* (Reference 3), compiled by default as **SplicePredictorLL**.

COMPILATION OPTIONS

The following parameters are set in the file
GENESEQER/include/sahmt.h (change and re-compile depending on need and available memory):

MAXGLGTH - maximum length of genomic DNA segment for spliced alignment; default: 15000
MAXCLGTH - maximum length of cDNA/EST for spliced alignment; default: 8000
MAXPLGTH - maximum length of protein sequence for spliced alignment; default: 3000

FILES

GENESEQER/README
GENESEQER/bin
GENESEQER/data (examples)
GENESEQER/doc/SplicePredictor.1 (this file)
GENESEQER/doc/SplicePredictor.1 (this file)
GENESEQER/include
GENESEQER/src

SEE ALSO

GeneSeqer(1), SplicePredictorLL(1).

NOTES

A hardcopy of this manual page is obtained by 'man -t ./SplicePredictor.1 | lpr'.

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