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varSEAK Online

Splice Site Prediction

Version 2.0

developed by

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Table of Contents

1 Introduction

The JSI splice site prediction tool calculates splicing effects for genetic variants. It is available on **varSEAK Online** as well as in the **varSEAK** Software. The software requires canonical splice sites (core motif GT for 5' donor splice sites or AG for 3' acceptor splice sites). Non-canonical splice sites, e.g. core motif GC for 5' donor splice sites, are not taken into consideration.

We would like to thank Gene Yeo for his friendly approval to integrate the MaxEntScan scoring algorithm (Yeo & Burge, 2003) into our software.

Version 2.0 was developed to optimize the algorithm as well as the usability. The re-engineered algorithm has an accuracy of 96.4% (for more details, please see Development and validation, page 3). The application is more intuitive and comprehensible due to a clearer design that focuses on the relevant scores and interpretations.

1.1 Disclaimer

The information generated by the JSI splice site prediction tool is not intended for direct diagnostic use or medical decisionmaking without consulting a genetics professional. Users must be careful and well positioned to judge and verify the information made available before relying on it.
The information shown are results based on a trained prediction algorithm (see also Development and validation, page 3). Although the predictions show a very good hit rate when validated on sample data, they should under no circumstances be used without further confirmation. The predictions are generated with the latest algorithms, but can by no means replace evidence-based data from further investigations (wet lab).

2 Development and validation

The JSI splice site prediction algorithm was trained on approximately 200 000 real splice sites taken from GRCh37 and 300 000 false splice sites from the HS3D dataset (P Pollastro & Rampone, 2003; Pasquale Pollastro & Rampone, 2002). The resulting algorithm was tested for accuracy using a dataset by Leman et al., consisting of 395 variants with known splicing effect (Leman et al., 2018).
Accuracy is determined as (Number of correct assessments)/(Number of all assessments). With this dataset, the JSI splice site prediction algorithm has an accuracy of 96.4%.

3 Instructions for use

To display this web page we recommend the web browsers FireFox or Chrome. Note: Internet Explorer is not supported.

3.1 Analyze a single variant

To analyze a single variant, you can either enter the required information on the main page or you can change the input at the top of the results page.

3.1.1 Enter information

The information required is:

- a gene (type to narrow down the options available from the list)
- a transcript (a list of transcript will be offered once a gene has been selected. Identical transcripts from different sources will be listed together)
  - if no transcript is given, the longest available transcript for this gene will be used automatically
- a variant, which can either be
  - a c.-HGVS nomenclature (such as c.1585-8G>A)
  - a sequence (min. 20 up to 150 bases, we recommend at least 50 bases, the variant should approximately be at the center)

Click the *[Search]* or *[OK]* button to start the analysis.

To analyze another variant in the same gene, you can simply change the HGVS or sequence. You can also change the transcript using the arrow button behind it. If you wish to analyze a variant on another gene, click the list button behind the gene name. A modular window will open where you can change the input. Once you have selected another gene, you can again select your desired transcript. *[OK]* starts the analysis, *[Cancel]* lets you return without any changes.

3.1.2 Results

3.1.2.1 General information

The input box will be filled with details concerning the gene and transcript, such as chromosome, strand, start and end positions as well as exon number and cDNA length.

Below the input field for HGVS or sequence, details concerning the position of the variant (exon/intron, cDNA position, genomic position) are displayed.

3.1.2.2 Sequence graph and legend

At the top of the results, a sequence graph depicts the reference and variant sequence and important features. A legend is given below on the right hand's side in the "INFO" box.

The variant is always placed at the center of the shown sequence. It is highlighted in red, the HGVS nomenclature is given above.

Important authentic or potential splice sites are marked with numbered triangles corresponding to the table on the left hand's side below the sequence graph. Authentic splice sites are also highlighted in purple both in the graph as well as in the table on the left. Cryptic splice sites predicted to be activated are highlighted in pink.

3.1.2.3 Overall predicted class

The overall prediction is given as a splice site prediction class in the center of the page. Possible classes are:

- Class 1 (dark green): No splicing effect
- Class 2 (light green): Likely no splicing effect
- Class 3 (yellow): Unknown splicing effect
- Class 4 (orange): Likely splicing effect
- Class 5 (red): Splicing effect

The overall predicted class is always the highest occuring class of all listed results for both 3' and 5' splice sites, should both be influenced.

3.1.2.4 Table with relevant splice site positions

To the left of the overall predicted class and below the sequence graph, there is a table listing relevant splice site positions with their individual SSP classes and scores.

Above the table, there is a button labeled either *[SSP 3']* or *[SSP 5']*, depending on which of both is displayed. If predictions for both are available, you can use these buttons to switch results for which type of splice site should be displayed.

If there is an authentic or an activated cryptic splice site shown in the sequence graph, the corresponding row of the table will be marked in the corresponding colour.

For each listed position, there will be the following information:

- splice site prediction class: 1-5 (see Overall predicted class, page 4, for more details)
- Score: Likelihood that this variant is predicted to be functional (positive values) or not functional (negative values), reaching from -100% to +100%. For splice sites that are as likely to work as they are not to work, the score is 0 %.

- ΔScore (DeltaScore): the difference between the Score of the splice site on the reference sequence and the Score of the splice site on the variant sequence.
- MaxEntScan: The ENT score from MaxEntScan (Yeo & Burge, 2003) is displayed alongside to compare.
- ΔMaxEntScan (DeltaMaxEntScan): the difference between the MaxEntScan score of the splice site on the reference sequence and the MaxEntScan score of the splice site on the variant sequence.

For 3' splice sites, each row in the table can be selected with a bullet point, changing for which 3' splice site the details (selected 3' ss, AG Exclusion Zone and Branch Point/ U2) should be displayed.

3.1.2.5 Prediction details

To the right, details for the corresponding prediction are displayed. If there are results both for 5' and 3' splice sites, the details will be displayed for the selected splice site type. The overall prediction class will not change, since it always is the worst occuring class for both types of splice sites.

3.1.2.6 Public DB Info

Below the prediction details, the most important information from the public databases is displayed in a small table.
You can view (if available for the corresponding variant): the rs Number, the varSEAK Classification, the ClinVar Clinical Significance and the gnomAD AF. Each available information is linked out in the same way as on the Variant Table.
If you wish to find more details, click the link below the table or at the top of the page, leading you to the varSEAK Online Variant Table with all results for your selected gene. Use "Refine Results" at the top of the page to search.
If none of the four fields from public databases is available, the table will not be displayed.

3.1.3 Export result

To export the prediction for the given variant, click the button *[Export to PDF]* on the upper right hand's side, above the sequence graph. A PDF file will be generated and offered to you for download.
The report will contain the gene, transcript and variant als well as the overall and detailed predictions for the positions and scores of interest.

3.2 Analyze file

You also have the option to analyze a file containing more than one variant.

3.2.1 Select and upload file

Click the button *[Analyze File]* on the upper right hand's side, above the sequence graph. A modular window will open, listing the specifications required in terms of file format. At the bottom, you can choose to *[Close]* the Window (as well as with the *[X]* button in the upper right corner), or you can click *[Choose and analyze file]*. This in turn will open the file explorer for you to select and

upload a file. You will be prompted to wait while the file is analysed and a PDF report is generated. The report will then be offered to you as a download.

3.2.2 Result format

The report consists of a list of your variants, ordered by gene. For each variant, the SSP Class is given, together with the most important prediction details.

4 Examples

#	Gene	HGVS	Class	Prediction details	Actual effect	Reference
1	ABCA1	NM_005502.3 c.1195-27G>A	5	3' Acceptor Site: Use of de novo Splice Site 25 nt upstream of 3' ss Pos 1195-27 : De novo AG in AG-Exclusion-Zone.	25bp of intron 10 included in transcript	(Fasano et al., 2012)
2	BRCA1	NM_007294.3 c.4484G>A	4	5' Donor Site: Likely loss of function for authentic Splice Site. Exon Skipping Pos 4484+1 : Decrease of Score.	Exon 14 skipped	(Leman et al., 2018)
3	BRCA1	NM_007294.3 c.5153-1G>A	5	3' Acceptor Site: Use of cryptic site 1 nt downstream of 3' ss Pos 5153-2 : No AG.	Use of a cryptic site 1 nt downstream from 3' ss	(Leman et al., 2018)
4	BRCA2	NM_000059.3 c.7807G>C	1	3' Acceptor Site: No splicing effect	No change	(Leman et al., 2018)
5	CERS3	NM_001290343 c.609+1G>T	5	5' Donor Site: Loss of function for authentic Splice Site. Exon Skipping Pos 609+1 : No GT.	ss abolished	(Radner et al., 2013)
6	CFTR	NM_000492.3 c.1585-9T>A	5	3' Acceptor Site: Use of de novo Splice Site 7 nt upstream of 3' ss Pos 1585-9 : De novo AG in AG-Exclusion-Zone. Pos 1585-2 : Loss of function for authentic Splice Site.	exon skipping and 7bp retention due to cryptic ss	(Sharma et al., 2014)
7	RHD	NM_016124.4 c.1074-2A>C	5	3' Acceptor Site: Use of cryptic site 13 nt downstream of 3' ss Use of cryptic site 13 nt downstream of 3' ss Pos 1074-2 : No AG.	exon 8 skipping of strong intensity and use of cryptic splice site at 13 nt downstream from 3' ss	(Leman et al., 2018)
8	SLC40A1	NM_014585.5 c.387C>T	2	5' Donor Site: Likely no splicing effect.	no change	(Leman et al., 2018)

5 References

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