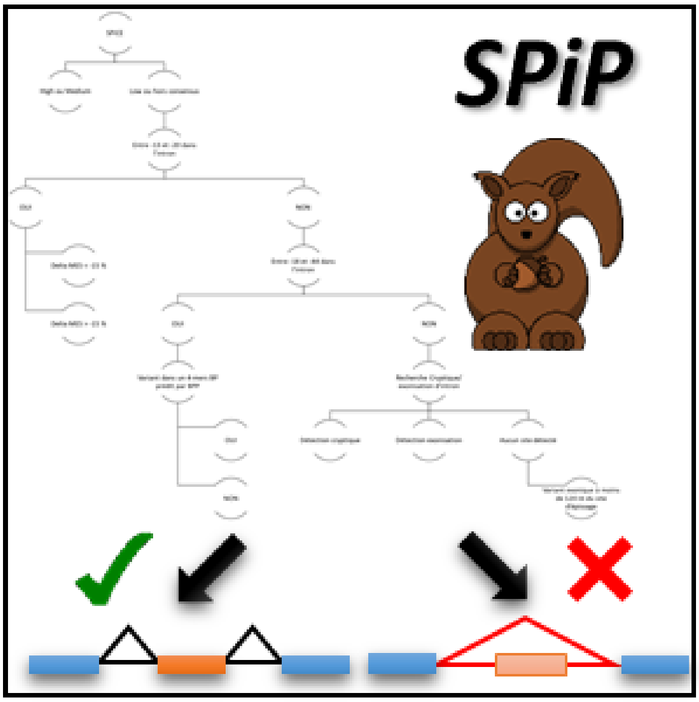
# SPiPv0.5

## (Splicing Prediction Pipeline)

Handbook



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# Contents

## INTRODUCTION

SPiP is a decisional tree running a cascade of bioinformatics tools. Briefly, SPiP uses SPiCE tool for the consensus splice sites (donor and acceptor sites), MES for polypyrimidine tract between -13 and -20, BPP for branch point area between -18 and -44, a homemade score to research cryptic/de novo activation and ΔtESRseq for exonic splicing regulatory element until to 120 nt in exon (see figure 1).

**Splicing prediction pipeline**

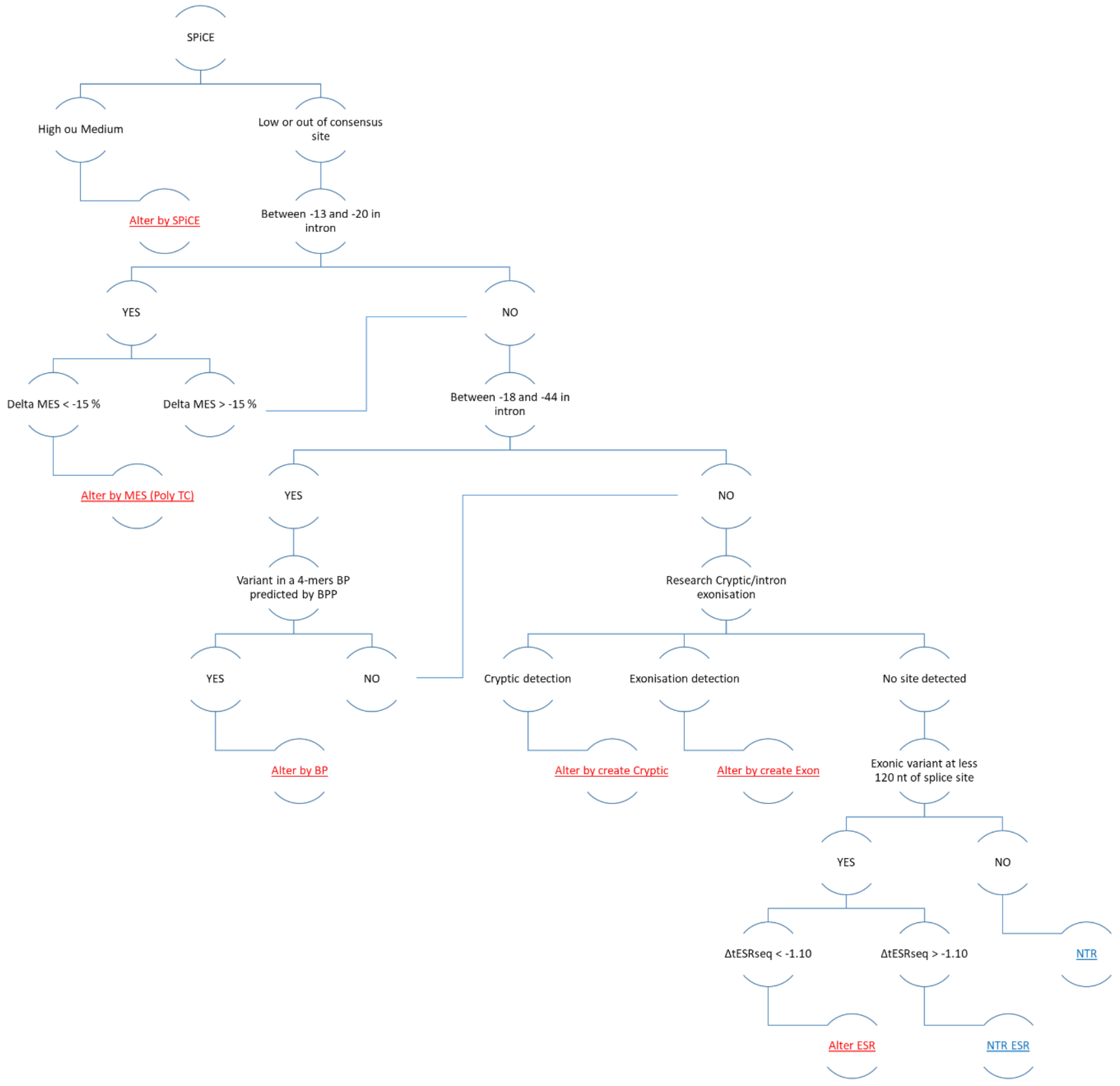


**Figure 1:** decisional tree of SPiP

For more information on SPiP development and rationale, please refer to the article: “SPiP: a Splicing Prediction Pipeline addressing the diversity of splice alterations, validated on a curated diagnostic set of 2,784 exonic and intronic variants.” (Raphaël LEMAN *et.al.*, article in progress).

SPiP has been developed in R langage (file “Rscript.R”), has embedded all score and database that it needs, and is freely available at: https://sourceforge.net/projects/splicing-prediction-pipeline/. To run variant, SPiP needs only the position and nucleotidic change either in text file or VCF file.

> ⚠
>
> The main of SPiP is to prioritize the RNA studies and **NOT** to predict the pathogenicity of this variant.

**SPiP FOR WINDOWS**

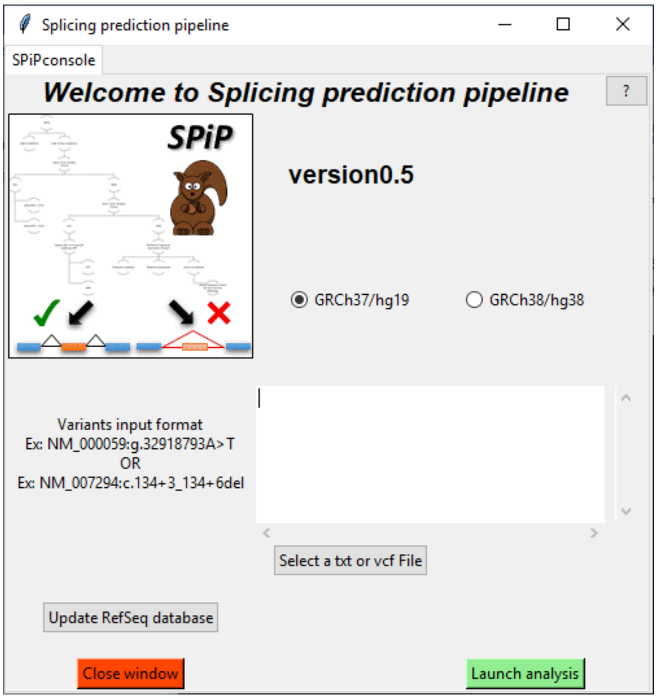
**SPiP installation**

SPiP is a portable software system. It is available in zip file or in executable format following your preferences. For zip file, after download, extract the files and open “SPiP.bat” to launch SPiP. For executable version, you have it in 32 and 64 bit windows versions. After download, double-click on the installer and follow the instruction. In this version a shortcut will be automatically created in your desktop.

A web connection is mandatory to request the sequences on the Ensembl database. If SPiP can’t connect to the EnsemblAPI, the program will be kill and the appropriate error message will appear in the prompt of windows console.

**SPiP running**

The SPiP console permits to select the genome version assembly, the import of variant and to set the options of SPiP.



Welcome to Splicing prediction pipeline

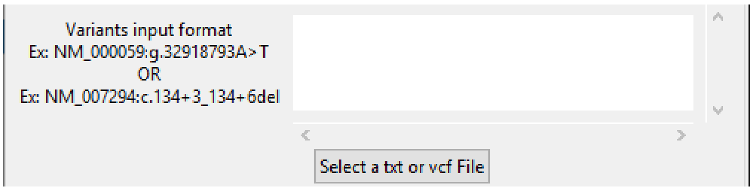
version0.5

● GRCh37/hg19 ○ GRCh38/hg38

Variants input format
Ex: NM_000059:g.32918793A>T
OR
Ex: NM_007294:c.134+3_134+6del

The “Update RefSeq database” button permits to update the RefSeq database (both hg19 and hg38) used by SPiP.

### Import of variants


Variants input format
Ex: NM_000059:g.32918793A>T
OR
Ex: NM_007294:c.134+3_134+6del

You can directly write the variations in text dialog or import a file (txt or VCF format) for a batch analysis. Excepted for VCF files, the variation must have the syntax “Transcrit:mutation”. The transcripts are named according to the RefSeq database (NCBI). You can enter your variant by its position either by gDNA or cDNA coordinates. See examples below:

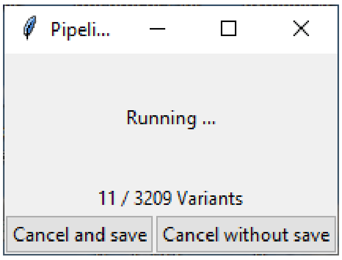
| Mutation type | Examples |
|---|---|
| Substitution | NM_000059:c.68-4A>G<br>NM_007294:g.41251855:G>T |
| Deletion | NM_007294:c.134+3_134+6del<br>NM_133509:g.68353824_68353826del |
| Duplication | NM_007294:c.211dup<br>NM_007294:g.41258474dup<br>NM_000059:c.9501_9501+1dup |
| Insertion | NM_058216:c.835+5insAAC<br>NM_024675:g.23653392insCGT |
| Deletion/Insertion | NM_032043:c.-30-3_-29delinsTTC<br>NM_002878:g.33445643_33445640delinsTT |

To import a variant in a txt file, the table must be tabulate separated, and you have to indicate ‘varID’ as the name of the column with the variant positions. A file example is providing at ‘testCrypt.txt’.

For VCF file format, SPiP supports version 4 or later as shown in the example file ‘testVar.vcf’. For each line, SPiP will check all transcript aligned on the mutation position. If SPiP didn’t find a transcript matching with the position of the mutation, this mutation will be not analyzed. A list of mutation exclude to this issue is display at the begin of SPiP running in the prompt of windows (“I find no transcript for the mutation(s): …. “).

### Runtime of SPiP

SPiP progression:



Running ...

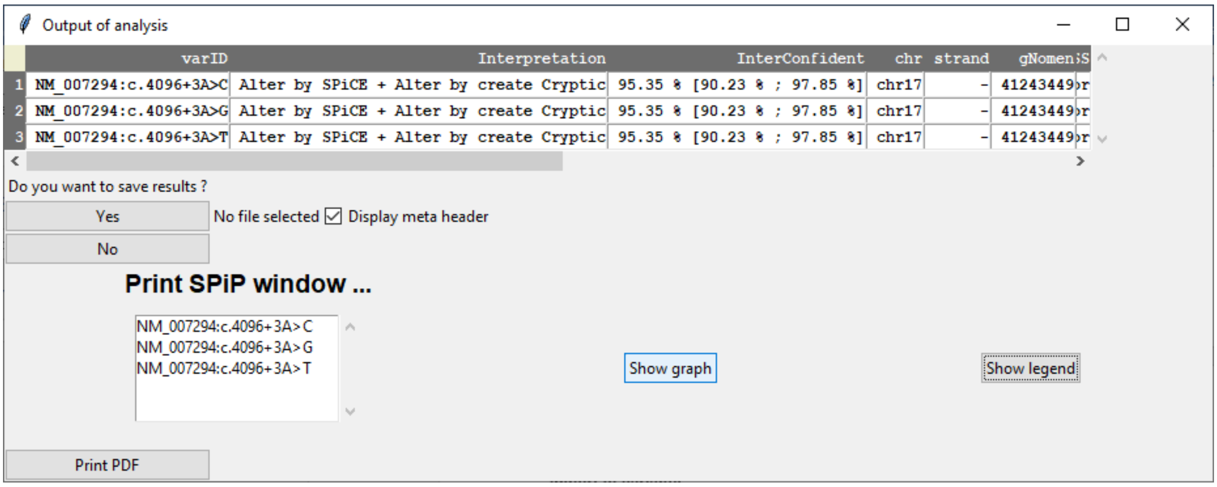
11 / 3209 Variants

The “Cancel and save button” permits to stop the program and automatically save the current result in a text file with random name in the folder of import data.

With an AMD Ryzen 7 PRO 1700 Eight-Core processor 3.00 GHz and 16 Go of RAM, SPiP toke 30-35 minutes to analyze 3,200 variants.

**SPiP Output**

After score calculation, SPiP opens the following window:



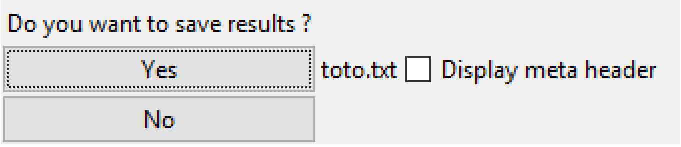
Output of analysis

| | varID | Interpretation | InterConfident | chr | strand | gNomen;S |
|---|---|---|---|---|---|---|
| 1 | NM_007294:c.4096+3A>C | Alter by SPiCE + Alter by create Cryptic | 95.35 % [90.23 % ; 97.85 %] | chr17 | - | 41243449›r |
| 2 | NM_007294:c.4096+3A>G | Alter by SPiCE + Alter by create Cryptic | 95.35 % [90.23 % ; 97.85 %] | chr17 | - | 41243449›r |
| 3 | NM_007294:c.4096+3A>T | Alter by SPiCE + Alter by create Cryptic | 95.35 % [90.23 % ; 97.85 %] | chr17 | - | 41243449›r |

Do you want to save results ?

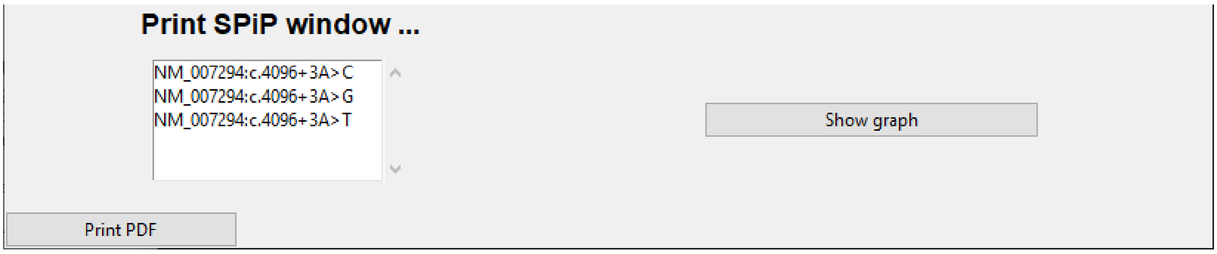
The check box ‘Display meta header’ is an option to add the description of each column generated by SPiP, uncheck it if you want only the results.

After save the results, the text at right of ‘Yes’ button will change to ‘No file selected’ to the name of file with results, here ‘toto.txt’.

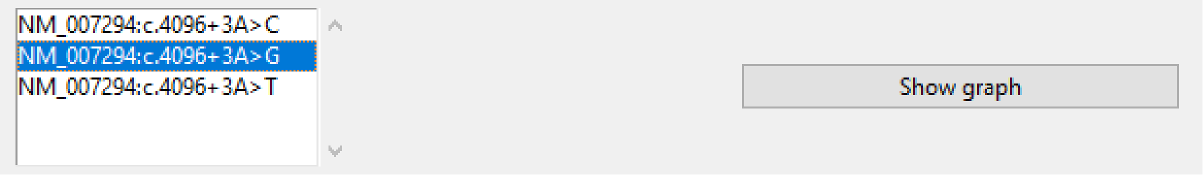


Do you want to save results ?

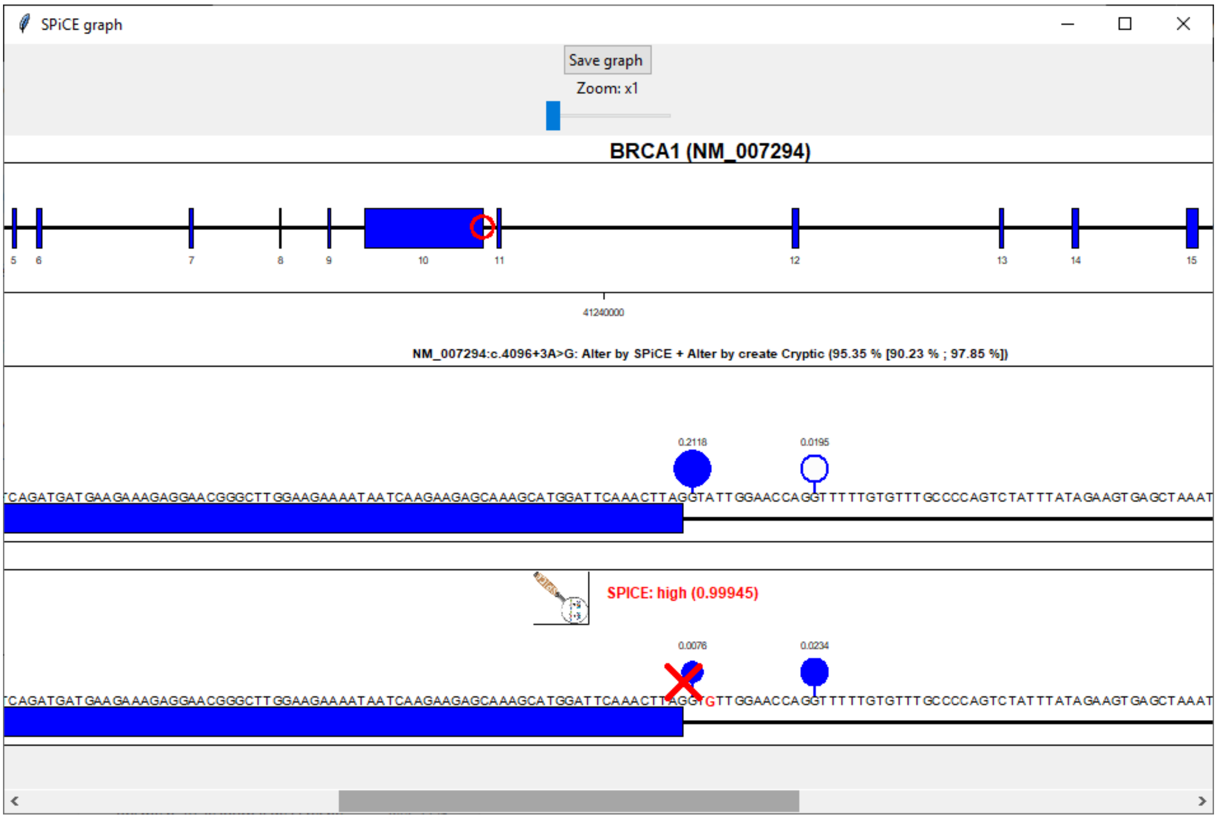
SPiP permits also to display the results in graphic format in the section:



Firstly, you can select a variant and press the button “Show graph”, here the variant NM_007294:c.4096+3A>G was selected:



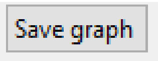
By pressing the button “Show graph”, the following window will open:


SPiCE graph
Save graph
Zoom: x1
BRCA1 (NM_007294)
5
6
7
8
9
10
11
12
13
14
15
41240000
0.2118
0.0195
TCAGATGATGAAGAAAGAGGAACGGGCTTGGAAGAAAATAATCAAGAAGAGCAAAGCATGGATTCAAACTTAGGTATTGGAACCAGGTTTTTGTGTTTGCCCCAGTCTATTTATAGAAGTGAGCTAAAT
0.0076
0.0234
TCAGATGATGAAGAAAGAGGAACGGGCTTGGAAGAAAATAATCAAGAAGAGCAAAGCATGGATTCAAACTTAGGTGTTGGAACCAGGTTTTTGTGTTTGCCCCAGTCTATTTATAGAAGTGAGCTAAAT

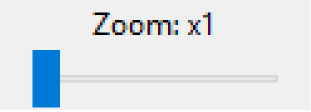
NM_007294:c.4096+3A>G: Alter by SPiCE + Alter by create Cryptic (95.35 % [90.23 % ; 97.85 %])

SPICE: high (0.99945)

You can use the button “Save graph” to save the graphic in PDF file.

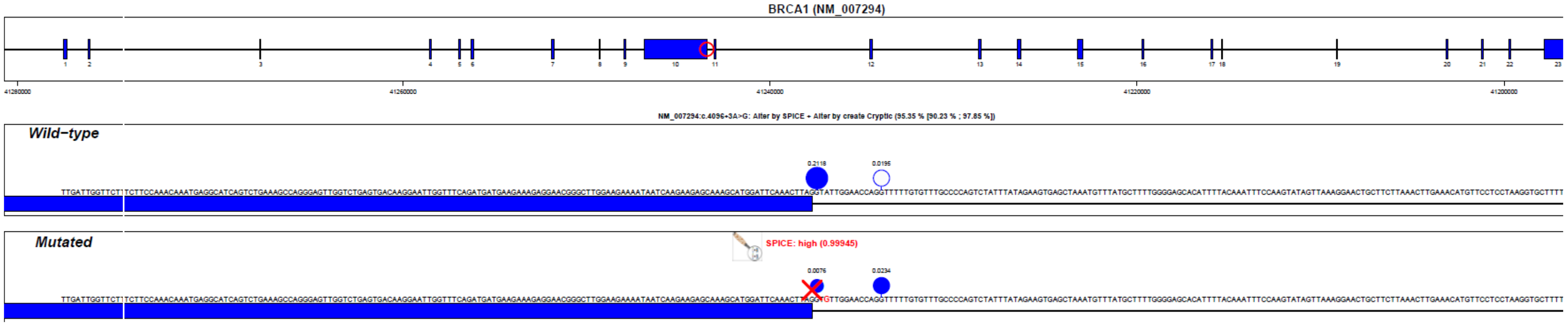


You can also enlarge the picture by the scale bar:

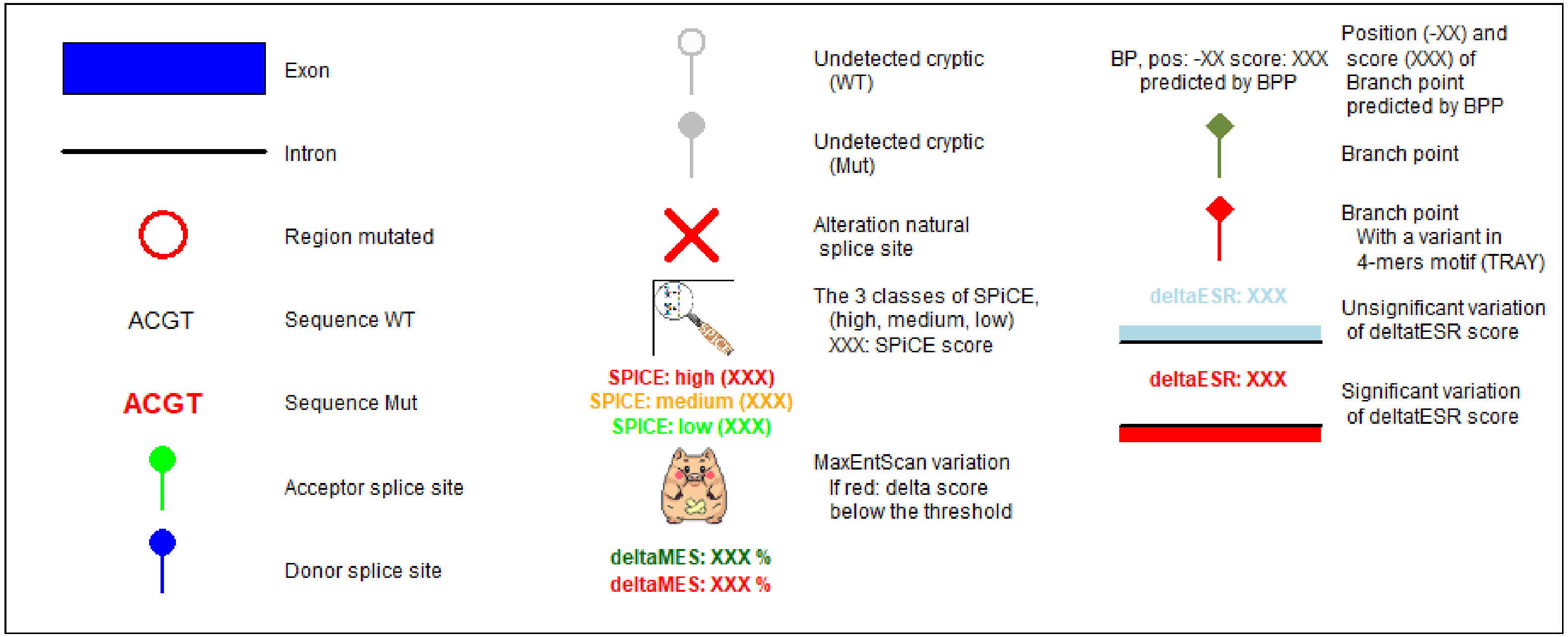


Secondly, you can also save the graphics for the entire analyzed variants by the button:

Example of graphic result in PDF file:

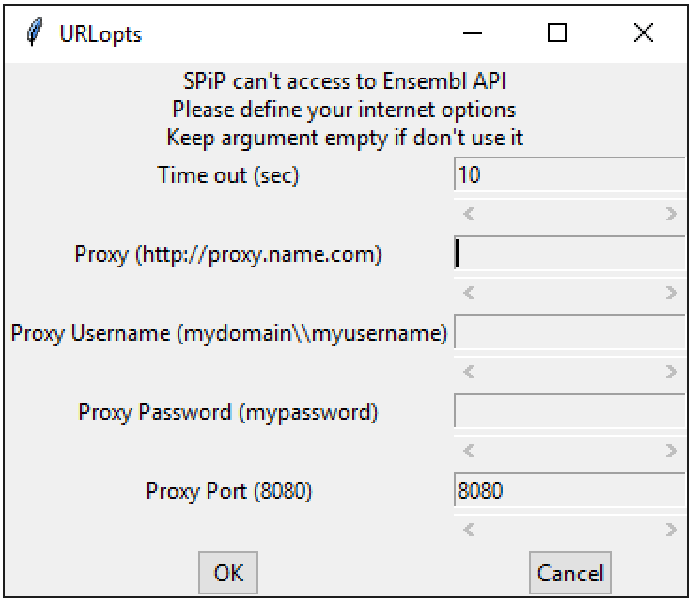

BRCA1 (NM_007294)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
41280000
41260000
41240000
41220000
41200000
NM_007294:c.4096+3A>G: Alter by SPICE + Alter by create Cryptic (95.35 % [90.23 % ; 97.85 %])
Wild−type
0.2118
0.0195
TTGATTGGTTCTTTCTTCCAAACAAATGAGGCATCAGTCTGAAAGCCAGGGAGTTGGTCTGAGTGACAAGGAATTGGTTTCAGATGATGAAGAAAGAGGAACGGGCTTGGAAGAAAATAATCAAGAAGAGCAAAGCATGGATTCAAACTTAGGTATTGGAACCAGGTTTTTGTGTTTGCCCCAGTCTATTTATAGAAGTGAGCTAAATGTTTATGCTTTTGGGGAGCACATTTTACAAATTTCCAAGTATAGTTAAAGGAACTGCTTCTTAAACTTGAAACATGTTCCTCCTAAGGTGCTTTT
Mutated
SPICE: high (0.99945)
0.0076
0.0234
TTGATTGGTTCTTTCTTCCAAACAAATGAGGCATCAGTCTGAAAGCCAGGGAGTTGGTCTGAGTGACAAGGAATTGGTTTCAGATGATGAAGAAAGAGGAACGGGCTTGGAAGAAAATAATCAAGAAGAGCAAAGCATGGATTCAAACTTAGGTGTTGGAACCAGGTTTTTGTGTTTGCCCCAGTCTATTTATAGAAGTGAGCTAAATGTTTATGCTTTTGGGGAGCACATTTTACAAATTTCCAAGTATAGTTAAAGGAACTGCTTCTTAAACTTGAAACATGTTCCTCCTAAGGTGCTTTT

## Legend


Exon
Intron
Region mutated
ACGT
Sequence WT
ACGT
Sequence Mut
Acceptor splice site
Donor splice site
Undetected cryptic (WT)
Undetected cryptic (Mut)
Alteration natural splice site
The 3 classes of SPiCE, (high, medium, low) XXX: SPiCE score
SPICE: high (XXX)
SPICE: medium (XXX)
SPICE: low (XXX)
MaxEntScan variation If red: delta score below the threshold
deltaMES: XXX %
deltaMES: XXX %
BP, pos: -XX score: XXX predicted by BPP
Position (-XX) and score (XXX) of Branch point predicted by BPP
Branch point
Branch point With a variant in 4-mers motif (TRAY)
deltaESR: XXX
Unsignificant variation of deltatESR score
deltaESR: XXX
Significant variation of deltatESR score

### Internet connection

SPiP needs an internet connection to access at the Ensembl API. Also at each SPiP running, the software checks the internet connection. In the case of SPiP can't access to internet the software proposes to define the parameters to get internet connection, to known the proxy information.



URLopts

SPiP can't access to Ensembl API
Please define your internet options
Keep argument empty if don't use it

Time out (sec): 10

Proxy (http://proxy.name.com):

Proxy Username (mydomain\\myusername):

Proxy Password (mypassword):

Proxy Port (8080): 8080

The Time out (sec) is the limit time to request the Ensembl API. Proxy is the address of the proxy of your institution. If the proxy needs login in, you can also define your username and the password. The last box is the port of proxy, by default is 8080.

## SPiP FOR LINUX

SPiP was also developed for a Linux environment. To install this version of SPiP besides the download of the SPiP package dedicated to Linux. SPiP needs an R environment with the libraries “Rcurl” and “parallel”. The installation of samtools with the human genome is preconize to improve the runtime of SPiP. Indeed, SPiP will use samtools to get the DNA sequences and no more an internet connection to get this sequence for each variant.

### Install SPiP

The command line to install and to launch SPiP is:

```
$ git clone https://github.com/raphaelleman/SPiP
$ cd ./SPiP
```

Install R libraries, from R console:

```
> install.packages(“Rcurl”)
> install.packages(“parallel”)
```

(Optional) Install samtools

```
$ wget https://github.com/samtools/samtools/releases/download/1.9/samtools-1.9.tar.bz2
$ tar xfvj samtools-1.9.tar.bz2
$ cd ./samtools-1.9
$ ./configure --prefix=/where/to/install
$ make
$ make install
$ # get the human genome (here from GENCODE website: https://www.gencodegenes.org/)
$ ## Warning: download the same version of genome that the genome version of your genomic
coordinates
$ wget
ftp://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_human/release_29/GRCh37_mapping/GRCh37.pr
imary_assembly.genome.fa.gz -O genomehg19.fa.gz # GRCh37/hg19 example
$ gunzip genomehg19.fa.gz
$ samtools faidx genomehg19.fa
```

### Run SPiP

Try the installation of SPiP with data sample:

```
$ cd /path/to/SPiP/
$ #without samtools
$ Rscript ./SPiPv0.5.r -I testCrypt.txt -O trySPiP.txt
$ #with samtools (+ hg19 assembly version)
$  Rscript  ./SPiPv0.5.r  -I  testCrypt.txt  -O  trySPiP.txt  -g  hg19  -s  /path/to/samtools  -f
/path/to/fastaGenome
```

The detailed options of SPiP are:

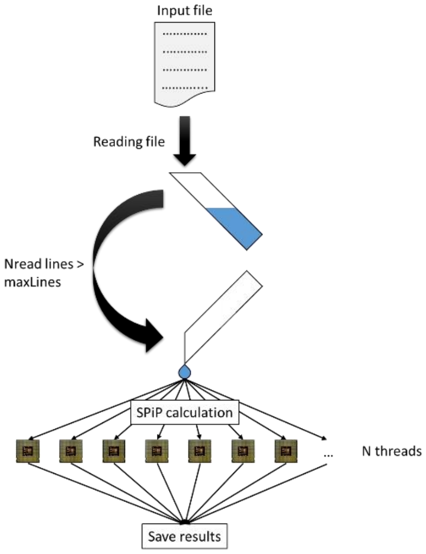
```
Usage: SPiPv0.5.r

    Mandatory

        -I, --input /path/to/inputFile      list of variants file (.txt or .vcf)
        -O, --output /path/to/outputFile    Name of ouput file (.txt)

    Genome options

        -g, --GenomeAssenbly hg19    Genome assembly version (hg19 or hg38) [default= hg19]
        -s, --SamPath /path/to/samtools]     Path to samtools, if you want to use Ensembl api
keep this argument empty
        -f, --fastaGenome /path/to/fastaGenome      fasta file of genome used by samtools

    Parallel options

        -t, --threads N      Number of threads used for the calculation [default= 1]
        -l, --maxLines N     Number of lines read in each time [default= 1000]

    Other options

        --header      Print meta-header info
    -h, --help   Print this help message and exit

   You could : Rscript SPiPv0.5.r -I ./testCrypt.txt -O ./outTestCrypt.txt
```

*SPiP and parallel analysis*

RunTime of SPiP

With an AMD Ryzen 7 PRO 1700 Eight-Core processor 3.00 GHz and 16 Go of RAM, SPiP toke 8-9 minutes to analyze 3,200 variants by using samtools. Without samtools, SPiP toke 30-35 minutes to analyze the number of variants on the same machine.

During the parallel analysis, SPiP will read a part file, defined by the parameter `-l, --maxLines`, in the aim to load all file in the RAM memory. For each step, the read lines are analyzed by SPiP using the number of CPUs set by the parameter `–t, --threads`. See the illustration below:



## RESULTS OF SPiP

| Column header | Explanation |
|---|---|
| Interpretation | Global interpretation of SPiP |
| InterConfident | Confident of interpretation, ie the risk of splicing alteration |
| chr | Chromosome of the mutation |
| strand | Strand of the mutation |
| gNomen | Genomic position of the mutation |
| seqPhysio | A, C, G, T sequence of wild-type DNA |
| seqMutated | A, C, G, T sequence of mutated DNA |
| NearestSS | The nearest natural splice site type (5'/3' ss) of the mutation |
| distSS | Relative distance between mutation and splice site |
| RegType | Type of region where mutation occurring |
| SPiCEproba | Score of SPiCE |
| SPiCEinter_2thr | Interpretation class of SPiCE |
| deltaMES | MES variation score between wild-type and mutated sequences |
| mutInBParea | Mutation in Branch point |
| deltaESRscore | ESRseq variation score between wild-type and mutated sequences |
| posCryptMut | Position of the strongest cryptic site reinforced by the mutation |
| sstypeCryptMut | Splice type of the cryptic site |
| probaCryptMut | Cryptic score |
| classProbaCryptMut | Class of cryptic site |
| nearestSStoCrypt | Nearest natural splice site type to the cryptic site |
| nearestPosSStoCrypt | Position of the nearest natural splice site to the cryptic site |
| nearestDistSStoCrypt | Distance between the nearest natural splice site and the cryptic site |
| posCryptWT | Position of the strongest cryptic site before the mutation |
| probaCryptWT | Score of the strongest cryptic site before the mutation |
| classProbaCryptWT | Class of the strongest cryptic site before the mutation |
| posSSPhysio | Position of the natural splice site |
| probaSSPhysio | Score of the natural splice site |
| classProbaSSPhysio | Class of the natural splice site |
| probaSSPhysioMut | Score of the natural splice site after the mutation |
| classProbaSSPhysioMut | Class of the natural splice site after the mutation |

### SPiP global interpretation (columns: Interpretation, InterConfident)

In the column Interpretation, SPiP summarize the alteration detection of the mutations. The different ways of splicing alteration are annotated as follow:

| Annotation | Signification |
|---|---|
| Alter by SPiCE | The mutation alters the consensus splice sites |
| Alter by MES (Poly TC) | The mutation alters the polypyrimidine tract (-20 to -18) |
| Alter BP | The mutation alters a branch point |
| Alter by create Exon | The mutation creates a pseudo-exon |
| Alter by create Cryptic | The mutation creates a cryptic/de novo splice site |
| Alter ESR | The mutation alters the exonic splicing regulatory elements |
| NTR | The mutation has no effect on splicing |

When a mutation affects splicing by several ways, these ways are display together. For example, a mutation that disturbs the consenus splice site and create a cryptic site, the column Interpretation will contain "Alter by SPiCE + Alter by create Cryptic".

In the column InterConfident, the predictions are weighted by the risk of altering splicing accordingly the predictions and the position of mutation. These risk levels were estimated from a collection of 2,784 variants with their *in vitro* RNA study and 63,171 SNPs as control data. these different levels are illustrated in the next figure. In the case where a mutation a mutation is reported as two different effects, the risk displayed is the higher among the different way of alterations.

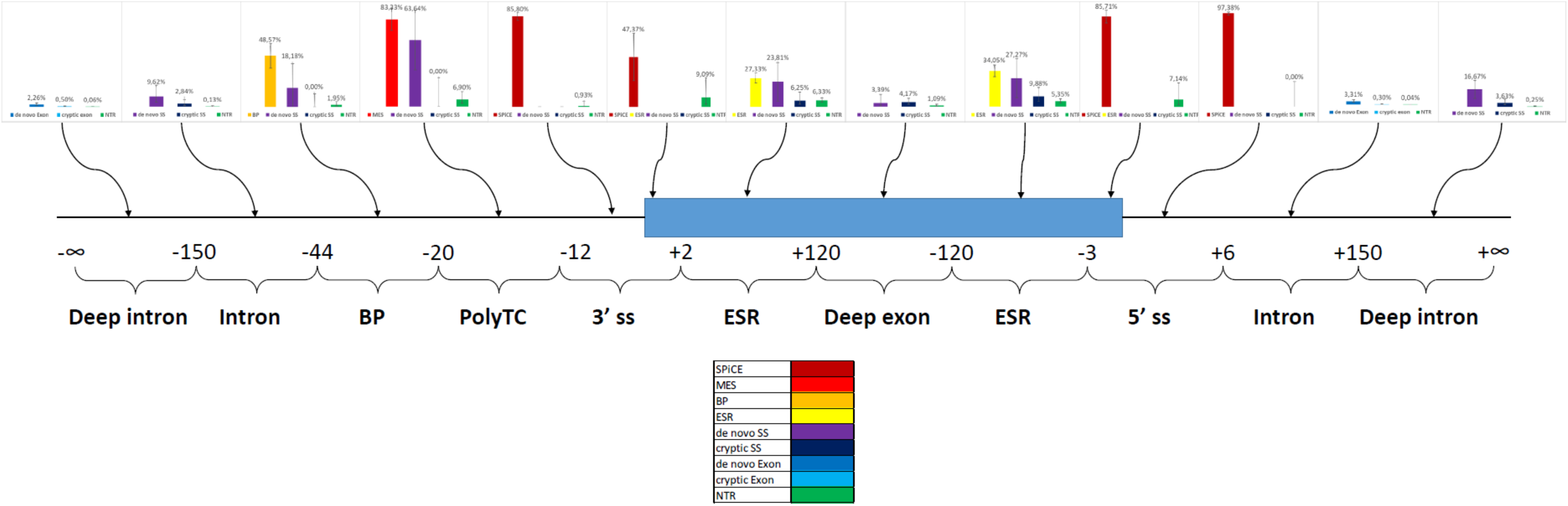
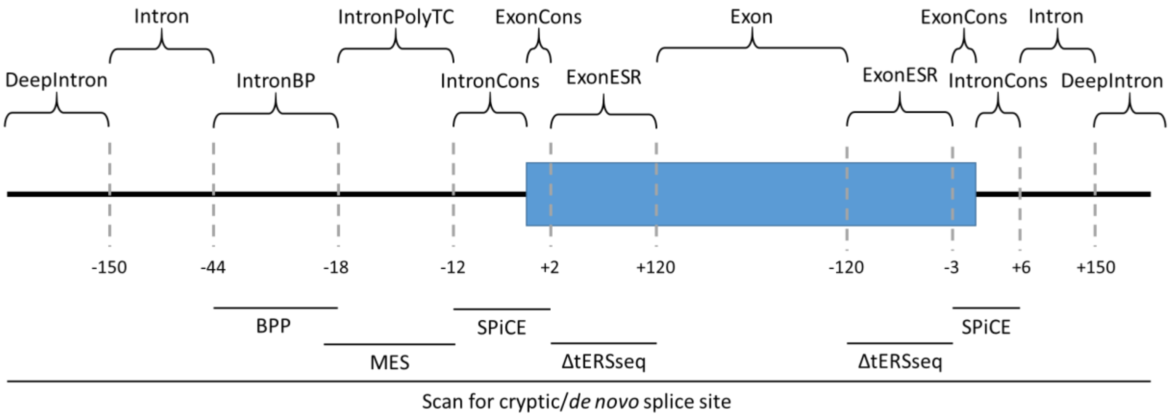


**Figure 2:** Probability of splicing alteration according the prediction and the localization of the mutation.

**General information (columns: chr, strand, gNomen, seqPhysio, seqMutated, NearestSS, distSS, RegType)**
The chromosome, strand and genomic coordinates are given accordingly the hg19 or hg38 assembly genome following your option choice at the begin of SPiP. The seqPhyio and seqMutated column contain the DNA sequences 150 nt around the position of mutation for the wild-type and mutated sequences respectively. The NearestSS and distSS columns contain the information about the nearest natural splice site. The RegType column gives information about the localization of mutation in the transcript.



**SPiCE tool (columns: SPiCEproba and SPiCEinter_2thr)**
SPiCE tool was used to score the consensus splice site acceptor/donor (3'ss/5'ss), defined as -12; +2 for 3'ss and -3; +6 for 5'ss. SPiCE displayed a score between 0 and 1. This score was used to class variant in three categories: low, medium and high. The categories medium and high are considered as impacting splicing and low-category as no impact on splicing. Any variant outside the consensus splice site are annotated with a score at 0 and class as Outside SPiCE interpretation.

**MES tool (column: deltaMES)**
MES tool was used for variant occurring in the polypyrimidine tract (defined from -20 to -12). A decreasing of score below -15 % of natural score is considered as an alteration of this tract and then an alteration of splicing.

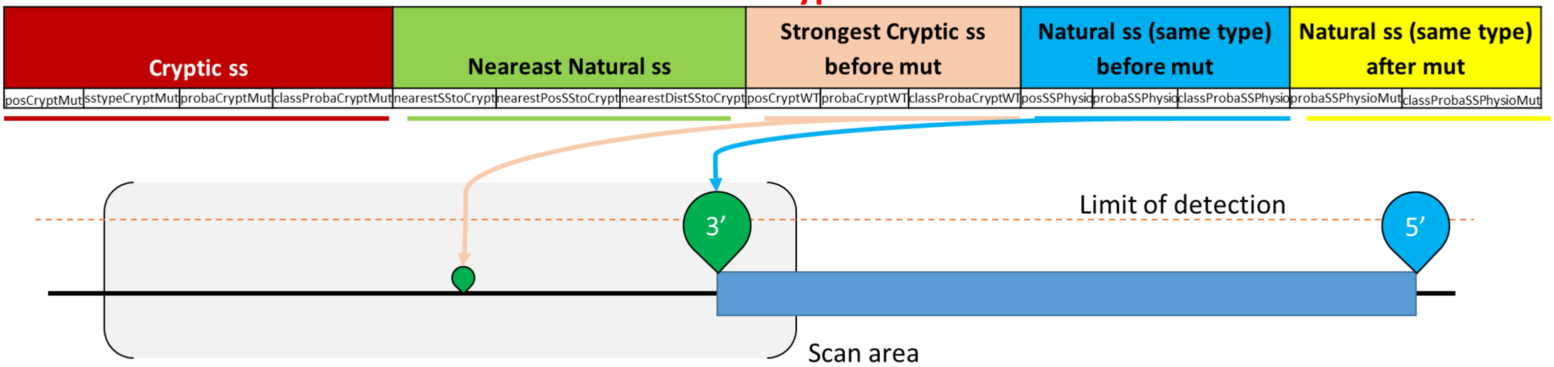
**BPP tool (column: mutInBParea)**
A comprehensive collection of BPP-predicted branch points was implemented in SPiP. We set one branch point (with maximal score) for each intron in transcripts described by RefSeq database. The variants located in branch point area (from -44 to -18) are aligned to this collection of branch points. If a variant occurs in the 4-mer of branch point (motif: TRAY), the variant is annotated as altering the splicing by branch point alteration. In this case the output is 'Yes g.29554209 (-27): 6.05': Yes: means that the the variant occurs in the 4-mer of branch point, 'g.29554209': the genomic coordinate of branch point, '(-27)' the relative distance to the natural 3'ss and '6.05': the score of BPP for this branch point.

**ΔtESRseq (column: deltaESRseq)**
For variants in exon and at least 120 nt of natural slice site are scored by ΔtESRseq. If variant decreases the score above -1.10, we consider it as disrupting the ESR motifs and then impacting the splicing.

**Scan for cryptic/*de novo* splice site (columns: posCryptMut to classProbaSSPhysioMut)**
Due to the complexity of cryptic/de novo splice site prediction, SPiP displays not only the score of cryptic splice site but also the score of the splice sites around the cryptic site. Briefly SPiP gives the score of cryptic sites reinforced by the mutation, the nearest natural splice site to this cryptic site, the score of strongest cryptic sites before the mutation and the score of nearest natural splice site of the same type than cryptic site before and after the mutation. To illustrate this, some examples were shown:

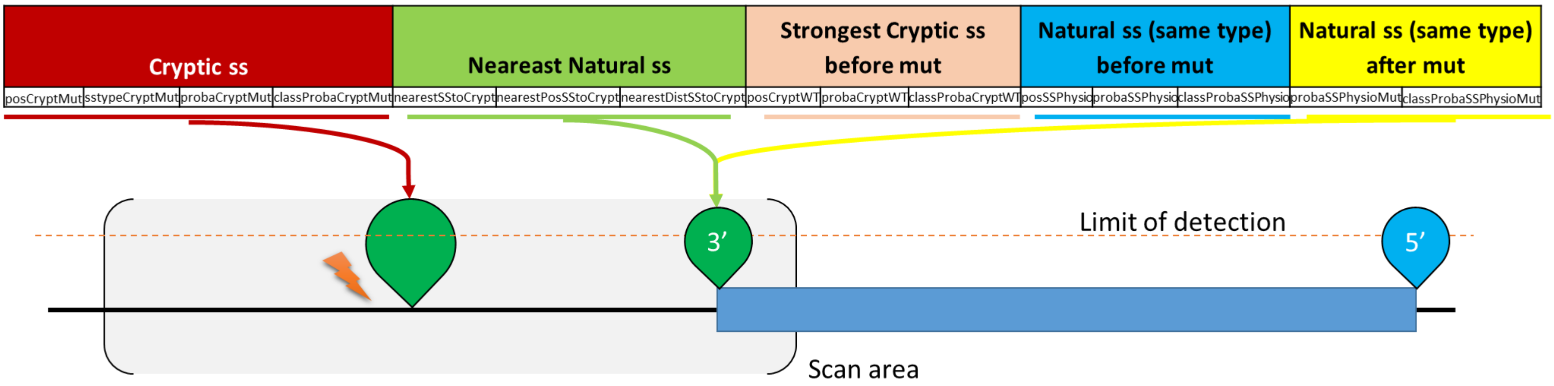
### Simple cryptic activation

**Wild-type**



| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |

**Mutated**

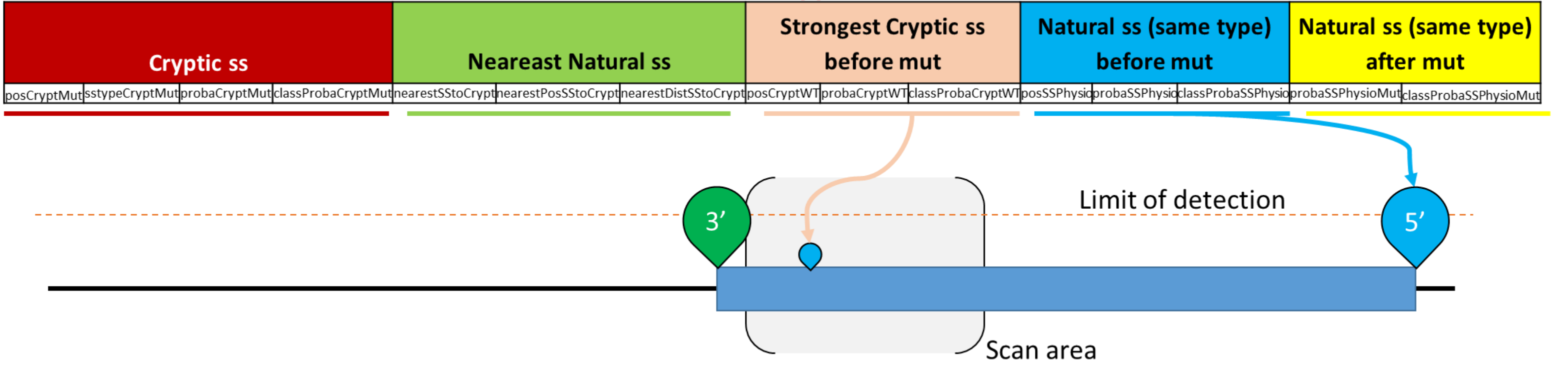


| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |

This is the simplest case, with a variant reinforcing a cryptic side beyond the threshold of cryptic detection.

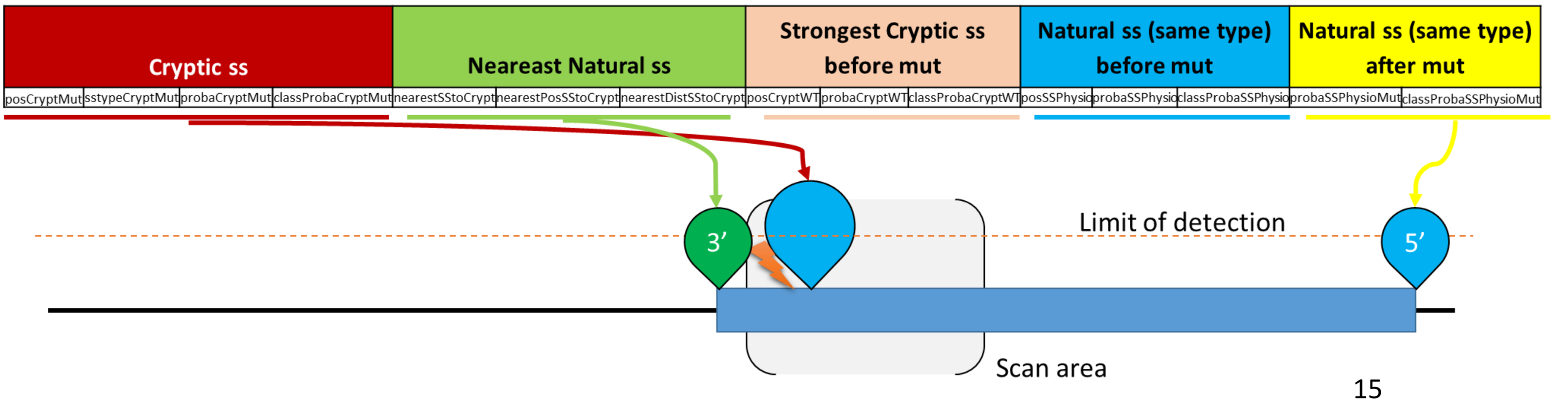
### Cryptic activation but two different nearest splice sites

**Wild-type**



| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |

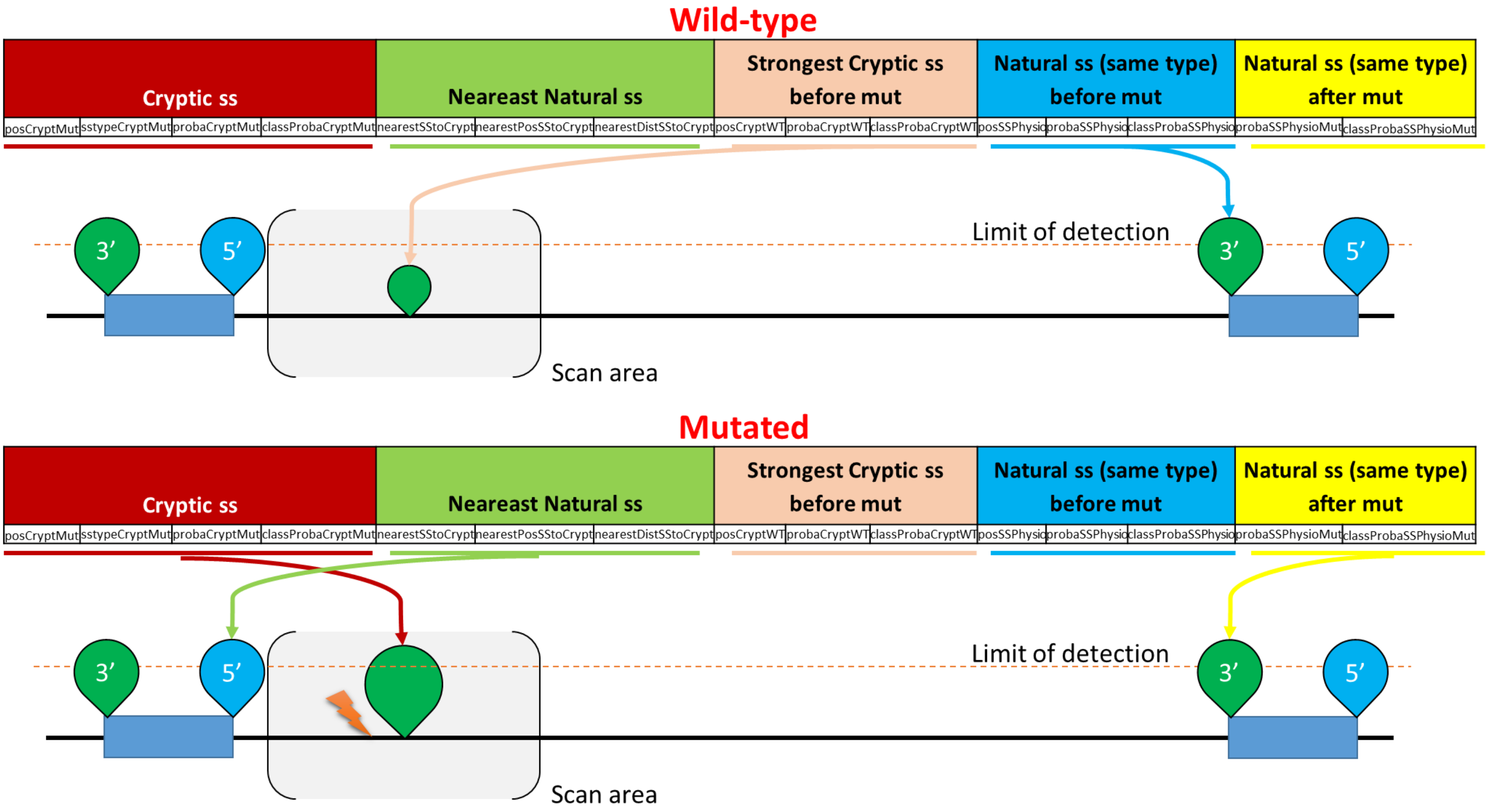
**Mutated**



| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |

The mutation active a donor cryptic site near to the natural acceptor splice site. So the nearest splice site was this acceptor site but the score displays by SPiP of the natural splice site was the score of the nearest donor splice site, to be coherent.

**SPiP selects the relevant spilce site**

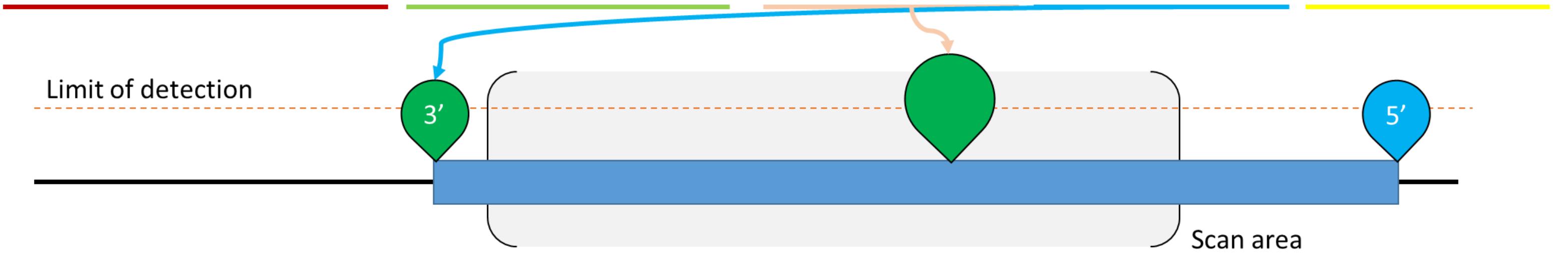


Here we have a similar configuration that in the precedent case. However, in this case, the cryptic site was an acceptor site and the nearest acceptor site was upstream of the nearest donor site. Also the use of this acceptor score to compare with the cryptic site score was not relevant. Thus SPiP toke the nearest acceptor site downstream of nearest donor site to take a relevant acceptor site as reference, even if this last was more far than the initial acceptor site.

**SPiP doesn’t takes systematically the stronger cryptic site**

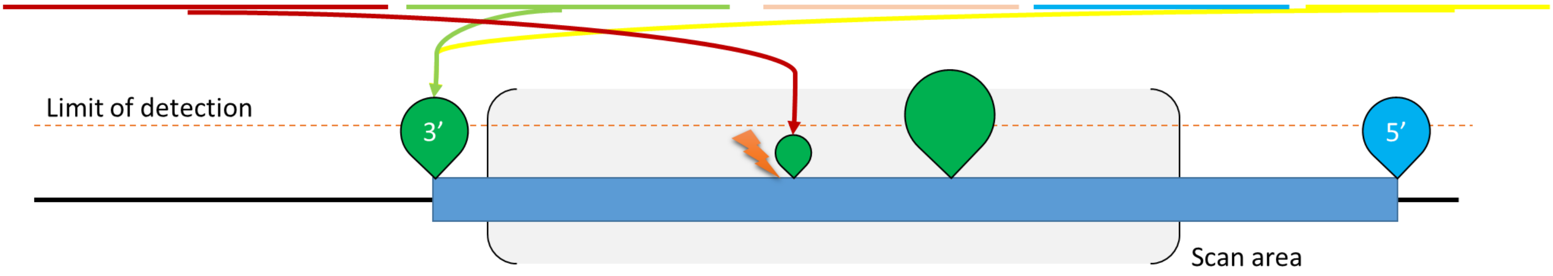
## Wild-type

| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |



## Mutated

| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |

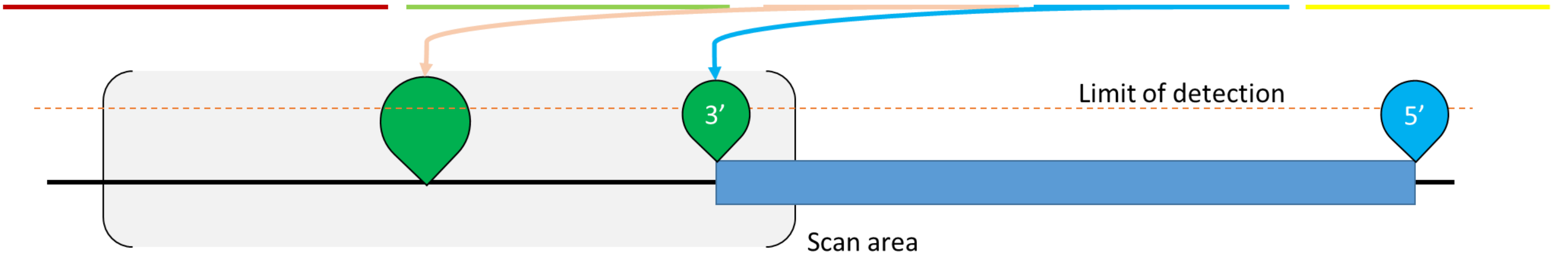


The mutated sequence shown two acceptor cryptic sites, the first was created by the mutation but below the detection threshold, the second was above the detection threshold but not impact by the mutation. SPiP displayed only the cryptic splice site impacted by the mutation even if the presence of stronger cryptic site and even if the mutation-impacted cryptic site was below the detection threshold.

### SPiP doesn’t take any cryptic site impacted by the variant

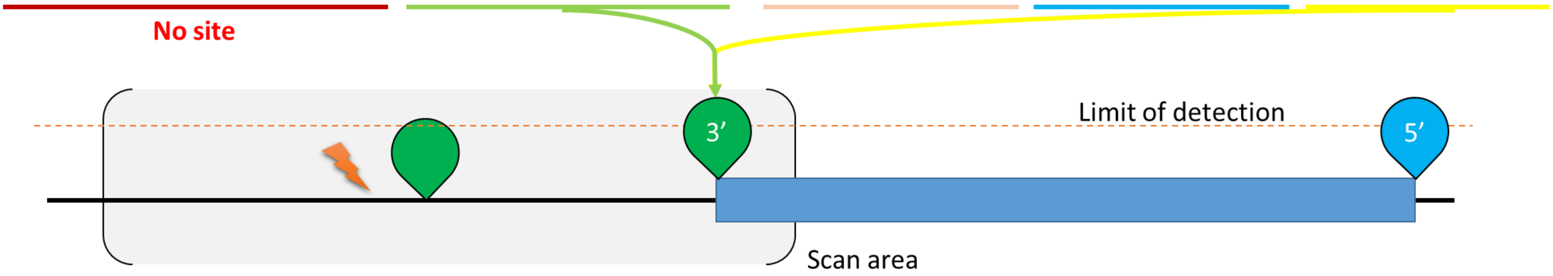
Wild-type

| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |



Mutated

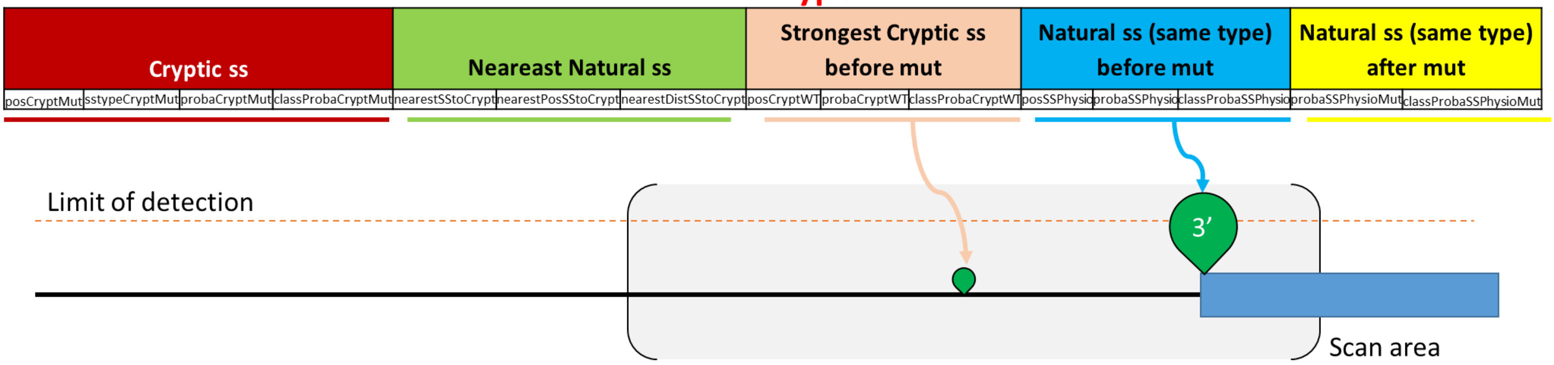
| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |



In this case, the mutation decreased the score of the acceptor cryptic site but this last remained above the limit of detection. SPiP didn’t display this site even if the score of this site was above the detection threshold.

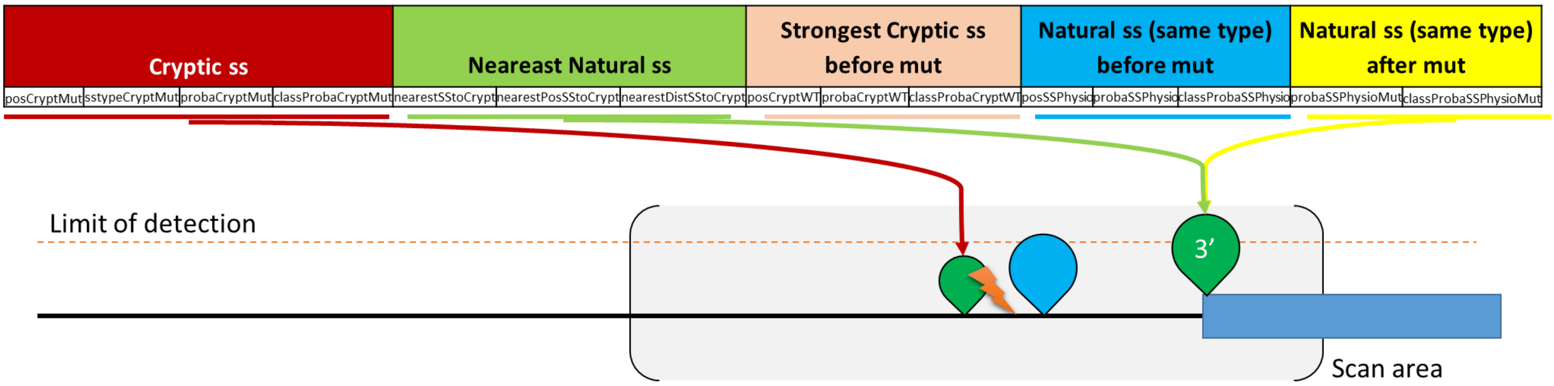
### SPiP doesn’t take any cryptic site impacted by the variant 2

Wild-type



| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |

Mutated



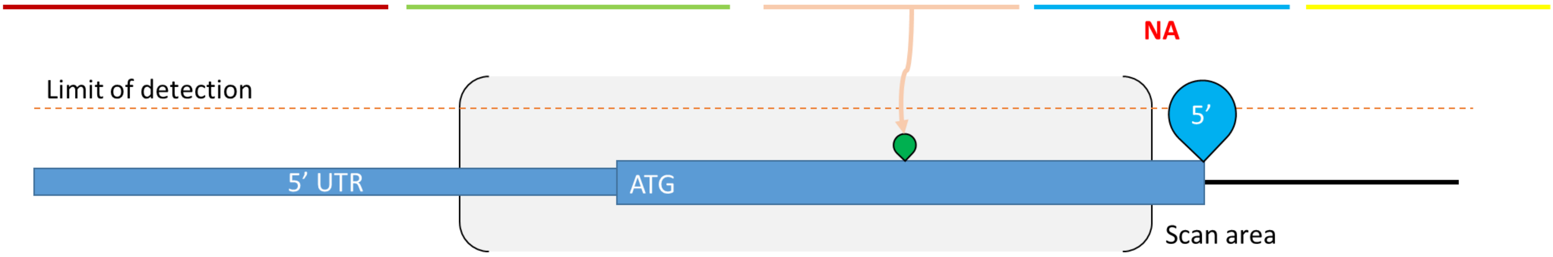
| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |

The mutation activated two cryptic splice sites, an acceptor and donor splice site. In the area of scan, SPiP found also a natural acceptor site. So SPiP toke the acceptor cryptic splice site even if this site had a lower score than the donor cryptic splice site. This filter works only if we have an natural splice site in the area of scan.

### The non-cassette exon case

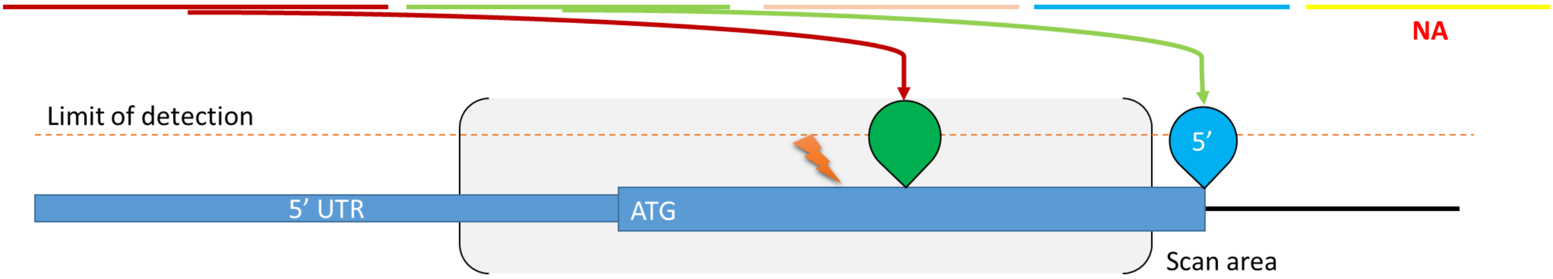
Wild-type

| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |



Mutated

| Cryptic ss | | | | Neareast Natural ss | | | Strongest Cryptic ss before mut | | | Natural ss (same type) before mut | | | Natural ss (same type) after mut | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| posCryptMut | sstypeCryptMut | probaCryptMut | classProbaCryptMut | nearestSStoCrypt | nearestPosSStoCrypt | nearestDistSStoCrypt | posCryptWT | probaCryptWT | classProbaCryptWT | posSSPhysio | probaSSPhysio | classProbaSSPhysio | probaSSPhysioMut | classProbaSSPhysioMut |



The mutation activated an acceptor cryptic splice near to the 5' UTR. In this case cryptic site was displayed but not the natural splice site to compare the score. SPiP shown instead a non-available data.

## ERROR MESSAGES AND RESOLUTIONS

| Error message | Description | Resolution |
|---|---|---|
| No file was selected! | No file selected while you clicked on button “Select a txt or csv File” | Select file in txt or in csv format |
| Incorrect format of input, please try again! | SPiCE cannot open your file | Check if your file is in txt or in csv format |
| I don't find the varID column, please try again! | SPiCE cannot find column with variant positions in your file | Name of variant position column must be “varID” |
| No input recording, please try again! | SPiCE was launched without sequences of consensus splice site | Save sequences of consensus splice sites either with dialog box or with file in txt or csv format |
| You must import the variant as: Transcrit:position nucleotidic change | There is an error in the variant position input format | You must enter variant position as Transcrit:position nucleotidic change e.g.: NM_000059:c.68-4:A>G |

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1. Burge: 20 Splicing of Precursors to mRNAs by the Spliceosomes [Internet]. [cited 2017 Sep 27]. Available from: https://cshmonographs.org/index.php/monographs/article/view/5123

2. Yeo G, Burge CB. Maximum Entropy Modeling of Short Sequence Motifs with Applications to RNA Splicing Signals. Journal of Computational Biology. 2004 Mar 1;11(2–3):377–94.

3. Shapiro MB, Senapathy P. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucleic Acids Res. 1987 Sep 11;15(17):7155–74.

4. Burset M, Seledtsov IA, Solovyev VV. SpliceDB: database of canonical and non-canonical mammalian splice sites. Nucleic Acids Res. 2001 Jan 1;29(1):255–9.