EUGÈNE: an open gene finder for eukaryotes and prokaryotes

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Overview

EuGène is a sophisticated open gene finder for eukaryotic organisms, and since version 4.0 for prokaryotic organisms also. It has been developed thanks to funding by INRA (permanent scientists and engineers), Génoplante and the french ministry of research (with one PhD student). It generates text, HTML and graphical outputs.

EuGène uses a graph based model to predict genes that covers both HMM based or more complex Bayesian net based or Conditional Markov Field probabilistic predictions. The model is fixed but complex (with 47 different states in eukaryote mode, and 64 in prokaryote mode) and covers Exon, Intron, UTR, UTR introns. . . each with a possible explicit distribution on length. The prediction itself relies on an optimal linear time and space algorithm for prediction.

Even if the gene model is fixed, the sources of information taken into account be EuGène for prediction are extremely varied and can be easily extended by creating so-called plugins. Currently, EuGène can use around 30 different plugins integrating statistical information (Markov models at DNA or amino acid level, WAM, Support Vector machine based signal prediction. . .), similarity information (Est, cDNA, proteins) and homology (exon conservation). It can also integrate predictions from other gene predictors if needed.

In order to integrate all this information, EuGène does not use maximum likelihood estimation for all parameters but parameters optimized by maximum of prediction quality on expertized data sets (minimizing empirical risk on a given dataset).

The software called `eugene` is written in C++ and is distributed under the artistic license.

Chapter 1

Reference documentation

To be executed, EuGène needs at least one file: this is the so-called parameter file. EuGène behavior is entirely controlled by a set of parameters whose default values are available in this file. These default values can be altered by editing this file or for some values through flags in the command line (such as `-d`). Command line flags override any value in the parameter file. The name of the parameter file that EuGène seeks is obtained by adding the suffix "`.par`" to the name of the EuGène command itself. As it is distributed, EuGène command's name is `eugene` and accordingly the parameter file is `eugene.par`. If at some point you want to use several different parameter files, you can simply use symbolic links to the `eugene` binary executable. Using a symbolic link, with a specific name to call EuGène will enable you to load a different parameter file whose name is derived from the symbolic link name by ading '`\.par`" The parameter file is first sought in the local directory. If this fails, the value of the environment variable `EUGENEDIR` is used as a second possible path.

EuGène gathers all informations on the FASTA sequences through so-called "plugins" also called "sensors". A plugin is a small software component that can be dynamically loaded and that can inform EuGène about likely exonic, intronic, utr, intergenic regions and about signals in the sequence (either splice sites, translation starts and stops, transcription starts and stops and possible frameshifts). Plugins can typically embody Markov models (that characterize exonic, intronic... regions) or splice site detectors or others. Available sensors are stored in the `PLUGINS` directory and are dynamically loaded by EuGène according to the parameters.

The typical call to EuGène is:

```
eugene <fasta files>
```

where each FASTA file contains one single DNA sequence. In this case, the first action of EuGène is to seek and load the parameter file. All the parameters in this file are either used by EuGène or by the plugins. Each plugin may have its own parameters. The following section describes all the parameters used by EuGène. Information about the parameters used by plugins is provided in each plugin section (see section 1.5).

1.1 EuGène's general parameters

Here is a list of all the parameters not related to a plugin which control EuGène's behavior. When a command line flag exists that can modify the corresponding parameter, it is indicated. All the parameters that control EuGène 's behavior are available in the parameter file. This file has a relatively strict formatting. Each line can either be a comment line (the first character in the line must be a #) or a parameter definition. Empty lines are not allowed. A parameter definition is composed of two strings of character. The first one is the name of the parameter, the second is its value. Everything is case sensitive. The definition order is not important.

- `EuGene.version`: specifies the EUGÈNE version. After having load the parameter file, EUGÈNE checks that the parameter file version is consistent with the executable version.
- `EuGene.organism`: name of the considered organism.
- `EuGene.mode`: 'Eukaryote' or 'Prokaryote'. The "`-P`" command line flag activates the Prokaryote mode. The value 'Prokaryote2' is also allowed (see section 1.2)
- `EuGene.sloppy`: in the default (non-sloppy) mode, EUGÈNE will stop and abort if some needed parameters in the parameter file is missing. If the parameter is set to `1` then a simple warning is emitted. Not advised unless you know what you do.
- `EuGene.VerboseGC`: If the parameter is set to `1` then display Garbage Collector Info.
- `EuGene.GCLatency`: latency of the garbage collector.
- `EuGene.ExonPrior`, `EuGene.IntronPrior`, `EuGene.InterPrior`, `EuGene.FivePrimePrior`, `EuGene.ThreePrimePrior`, `EuGene.RnaPrior`, `EuGene.BiCodingPrior`, `EuGene.UIRPrior`: prior on the initial/final state of prediction. The "`-s`" command line flag can override these priors by setting all the non intergenic priors to 0.0. This forces EUGÈNE to start and end its prediction in intergenic mode.
- `EuGene.InitExDist`, `EuGene.IntrExDist`, `EuGene.TermExDist`, `EuGene.SnglExDist`, `EuGene.IntronDist`, `EuGene.InterDist`, `EuGene.5PrimeDist`, `EuGene.3PrimeDist`, `EuGene.RnaDist`, `EuGene.OverlapDist`, `EuGene.UIRDist`: names of the files given explicit penalty distributions on the length. The path is relative to EUGENEDIR/models. EUGÈNE can use explicit penalty distributions on the length of the elements predicted. This can be an initial exon, and intermediary exon, a terminal exon, a single exon gene, an intron, an intergenic region, a 5' UTR region, a 3' UTR region or a non coding protein RNA. In prokaryote mode, this can be also an overlapping exons region or a untranslated intern region (UIR). Each parameter specifies the filename of an explicit penalty distribution file (see the following section).
- `EuGene.SplicedStopPen`: indicates the penalty for predicting genes containing in-frame spliced STOPs. This is basically set to an infinite value in order to avoid prediction containing spliced STOPs but setting this to 0.0 can be useful for pseudo-gene prediction...
- `EuGene.CodonTable`: name of the file which contains the DNA codon table. The path is relative to EUGENEDIR/models. This file is composed of three columns: the first contains the codon ; the second contains the amino acid (Just one letter) or the character '*' if it's a stop codon ; the third column contains the character '+' only if it's a start codon.

  Excerpt of the default eukaryote codon table:

  ```
  AAA K
  AAC N
  ACA T
  ...
  ATG M +
  ...
  TGA *
  ```
- `EuGene.NonCanDon`: list of allowed non canonical splice donor sites. (Separated by comma)
- `EuGene.NonCanAcc`: list of allowed non canonical splice acceptor sites. (Separated by comma)
- `Output.RemoveFrags`: in the text output, remove any fragmentary gene prediction (missing ATG or STOP or both). The prediction process is unchanged, the prediction is just filtered.

- `Output.truncate`: in the text output, each gene element predicted if prefixed by the FASTA sequence id (or the filename if no FASTA id is available). This is truncated to the number of caracters indicated. If set to 0 (or FALSE), the full id is used.
- `Output.MinCDSLen`: any predicted gene whose CDS length in number of nucleotides is lower than this is filtered out from the output.
- `Output.UTRtrim`: EuGene is natively capable of predicting UTR. If desired however, the UTR prediction of EuGene can be trimmed to be exactly consistent with the transcript evidence available as provided by the Est plugin. If no EST evidence is available, this means that all UTR predictions will be removed from the output.
- `Output.initid`: in the text output, initial value for numbering genes.
- `Output.stepid`: in the text output, step for numbering genes.
- `Output.graph`: if set, requests graphical PNG output. This can also be set using the `-g` command line flag. The PNG filename is composed by the seq name (w/o the .fasta suffix) completed by the number of the figure + .png extension (possibly, start/end positions will be inserted too if -u/-v is used).
- `Output.resx`, `Output.resy`: controls the horizontal and vertical resolution of the PNG images generated by EUGÈNE.
- `Output.gfrom`, `Output.gto`: respectively controls which part of the sequence is to be plotted (eg. for zooming). The default value for both is -1 which corresponds to the whole sequence. These parameters can also be set using the `-u` and `-v`. flags
- `Output.glen`: controls the number of nucleotides that will appear on a single image. The value -1 corresponds to a default adaptative mechanism which plots min (6000,length to visualize). The "length to visualize" is computed from the value given to `Output.gfrom` and `Output.gto`.
- `Output.golap`: controls how successives PNG images overlap. It must be set to the number of overlapping nucleotides between 2 successives PNG images. Default is -1 which heuristically determines this based on resolution and number of nuc. per image. This parameter can also be set using the `-c` command line flag.
- `Output.normopt`: indicates the way the score are normalized accross the possibles states (phase 1, 2, 3, -1, -2, -3, introns and intergenic states).
  - 0: no normalization
  - 1: normalize accross all states
  - 2: normalize each coding phase w.r.t. to the non coding score only.

  Default is 1. Does not affect prediction, only graphical output.
- `Output.window`: sets the half-size of the smoothing window used to plot the scores. Default is 48. This does not affect prediction, only graphical output. It can be set using the `-w` command line flag.
- `Output.intron`: allows to print introns in the textual output. Default is 0 (no introns).
- `Output.format`: controls the format of the textual outpout. May be `o` (stdout), `d` (detailed), `l` (long), `s` (short), `h` (html), `g` (gff) or `a` (araset format). Default is `l`. This can be overrided using the `-p` command line flag. `o`: print the prediction on stdout using the same format than `l`. All the others print the prediction in files which name are composed by the name of the sequence file (w/o the extension .fasta, .tfa, .fsa or .txt) completed by `.egn.debug (d)`, `.egn (l)`, `.egn.short (s)`, `.html (h)`, `.gff (g)`, `.gff3 (g)` or `.egn.ara (a)`. Multiple format can be selected (`ohg` for example). When GFF is requested, both GFF1 and GFF3 are produced.

- `Output.offset`: allows to offset the nucleotide position of the prediction. That is, the prediction for nucleotide at position i of the given sequence is printed as nucleotide $i+$ the offset. Useful to perform prediction on an extracted sequence without loosing the original position. Can also be set using the `-o` command line flag.
- `Output.Prefix`: indicates the directory where all non stderr/stdout output (eg. PNG images, HTML and GFF files...) should go. Default is the current directory.
- `Output.webdir`: location of the directory needed to generate html output. This location has to contain 'Image', 'Style' and 'Javascripts' directories. If the parameter is set to `LOCAL` then the web directory is EUGENEDIR/web. Else this parameter value has to be an URL.
- `Gff3.SoTerm`: indicates the path where sofa (Sequence Ontology Feature Terms) terms are store. The path is relative to EUGENEDIR.
- `Eval.offset`: during the evaluation of a prediction, the prediction is compared with a reference (the real gene structure). The region in which compare the prediction and the reference is defined as the reference positions +/- the offset. (used in optimization mode)
- `Eval.ignoreNpcRNA`: Put 1 to ignore the npcRNA for the fitness computing (used in optimization mode)
- `Fitness.wsng`, `Fitness.wsne`, `Fitness.wsnn`, `Fitness.wspg`, `Fitness.wspe`, `Fitness.wsspn`: indicate respectively the weight of the gene sensitivity, of the exon sensitivity, of the nucleotide sensitivity, of the gene specificity, of the exon specificity and of the nucleotide specificity in the fitness computing. (used in optimization mode)

Specification of explicit penalty distributions on length

As in semi-Markov models, EUGÈNE uses explicit distribution of penalties on the length of all predicted elements. The dynamic programming inside EUGÈNE garantees that EUGÈNE will run in linear time and space in the length of the sequence in all cases.

The distributions handled by EUGÈNE are made of 3 components. First, there is a region of forbidden length (minimum length), then a region with an arbitrary penalty distribution, then a region with a linear variation of the penalty. From a probabilitic point of view, this means an exponential tail.

Although EUGÈNE is linear in time in the sequence length, it is also typically linear in time in the sum of the size of the two first regions. For the moment, all existing EUGÈNE instances use explicit distributions with an empty arbitrary region (the distribution is just a minimum length followed by an exponential tail).

Explicit distributions must be specified in distribution files. Each line in a distribution file contains a length and a penalty. The first length used specifies the minimum allowed length. Then each line specifies a point of the explicit distribution. Linear interpolation is used between points. Then the last length used specifies the start of the linear tail. The last slope used becomes the slop of the linear tail.

A typical distribution file is given below:

```
3 0.0
4 2.0
6 4.0
```

It specifies a minimum length of 3. We then have an explicit distribution region with penalty 0.0 for 3, 2.0 for 4, 3.0 for 5 (linear interpolation), then 4.0 at 6. As this is the last point and the slope is 1, the rest of the distribution will be linear with slope 1.

1.2 EuGène's prokaryote mode

Since version 4.0, EuGène is able to annotate prokaryotic sequences. This mode is activated using the "`-P`" flag or equivalently by setting the parameter `EuGene.mode` to 'Prokaryote'. The value 'Prokaryote2' is also allowed: EuGène will do two independant predictions on the two strands. It is useful especially to predict antisense ncRNA.

In this mode, EuGène can predict overlapping genes on the same strand or different strands, and operon structure. Operon predictions are only available in the GFF3 output, activated by setting `Output.format` parameter to `g`.

Length constraints can be applied to overlap regions and to transcribed regions between genes, by filling respectively `EuGene.OverlapDist` and `EuGene.UIRDist` parameters.

Two genes predicted on the same strand whose distance is inferior to `Operon.maxDistance` are automatically consider as members of the same operon. `Operon.initid` indicates the initial value for operon numbering.

1.3 Splice variant prediction

Since version 3.4, EuGène allows to predict splice variants based purely on experimental data (alternative transcripts observed through EST, RNAseq or IsoSeq data). The feature is activated using the `-a` flag or equivalently by setting the parameter `AltEst.use` to 1 or TRUE.

In this case, EuGène will look for a file with the same name as the sequence file and with a suffix '`.alt.est`'. This file has the same format as the '`.est`' used by the Est pugin (see later) and contains information about genomic region with high quality similarity with EST. GFF3 format is allowed, by setting `AltEst.format` value to GFF3. The spliced alignment algorithm used to create this file should be of high quality, with clear exon-intron frontiers associated with splice sites.

Eugene only analyzes the EST alignments showing an inconsistency with a gene from the original prediction. That is to say the alignments where one of the exons shows at one of its borders a difference of at least `AltEst.IncompatibilityExonBorderMatchThreshold` nucleotides with an original gene.

EuGène will analyze the kept EST and try to produce a prediction that follows the EST structure. This prediction is performed in the region around the EST overlapping gene (+/- `AltEst.RepredictMargin` nucleotides). If the prediction is different from the optimal prediction (that is where one of its exons shows at one of its borders a difference of at least `AltEst.ExonBorderMatchThreshold` nucleotides with an original gene), the gene variant structure will be also output. Two files are created: one for the initial prediction (.gff3) and one also including the variants (.variants.gff3)

This feature is controlled by a number of other parameters with the '`AltEst`' prefix in the parameter file. The parameters that you could change are the parameters regarding length thresholds, used for filtering (`AltEst.maxEstLength`, `AltEst.minEstLength`, `AltEst.maxIn`, `AltEst.minIn`, `AltEst.maxEx` and `AltEst.minEx` which speak for themselves. These filters are applied if AltEst.extremeLengthFilter is activated.

If the ESTs are oriented, you can activate the parameter `AltEst.strandSpecific` to take into account the strand.

If `AltEst.includedEstFilter` is activated, EuGène will remove the EST alignments included in another. (Recommended use)

If `AltEst.compatibleEstFilter` is activated, EuGène will look for pairs of EST which are inconsistent one with the other (there is one nucleotide mapped to an exon by one which is mapped to an intron/gap by the other). Only EST of such a pair will be analyzed.

If `AltEst.unsplicedEstFilter` is activated, EuGène will remove the unspliced EST alignments.

Every alignment is also "trimmed" by an amount of `AltEst.exonucleasicLength` on the first and last hit to account for possible spurious short matches. If these hits are shorter than this amount, they are removed from the available data.

`AltEst.Penalty` is the penalty applied to each region incompatible with the EST alignment.

1.4 Splice variant prediction from a reference annotation

Since version 4.3, EuGène allows to predict variants from a reference annotation. Only splice variants of the reference genes would be predicted.

The expected format is GFF3 similar to the output of the egnep annotation pipeline. Note it only works if the egnep annotation was performed with `independent_strand_annotation`=0.

To load the reference annotation, fill in the GFF3 file using the `-k` parameter or equivalently by setting the parameter `AltEst.reference`. EuGene works as described in the section above and only the '.variants.gff3' file is created.

1.5 Plugins

Plugins are small software components that can be dynamically loaded by EuGène. Although it is completely transparent to the end-user, every plugin loaded by EuGène must be written in C++ and be a subclass of the Sensor class. This class provides essentially four methods:

- constructor: when instanciated, a plugin receives an instance number (specified in the parameter file) and a DNA sequence (instance of the `DNASeq` class). The instance number allows to load several identical plugins using different parameters. A plugin with a parameter `X` and instance number `n` will fetch parameter `X[n]` in the parameter file. On instanciation, the plugin should load all data needed to handle the sequence. If the plugin depends on optimizable parameters (parameters whose name is followed by a `*`), then the final configuration that may depend on these parameters must be postponed in the `Init` method.
- `Init`: receives as argument the sequence to process (an instance of the `DNASeq` class) and performs the extra initializations that depends on optimizable parameters values (parameters whose name is followed by a `*`).
- `GiveInfo`: receives as argument the sequence to process (an instance of the `DNASeq` class), a position on the sequence and a `Data` instance. The `Data` data-structure can receive predictions on all signals and contents scores known to EuGène.
- `Plot`: receives as argument the sequence to process (an instance of the `DNASeq` class) and plots all the predictions made by the sensor.
- `PostAnalyse`: receives as argument the prediction of EuGène and may check it against its own prediction and report support or inconsistencies.

The `Plot` and `PostAnalyse` methods are often empty. The `Init` is usually limited to the reloading of optimizable parameters (see the source of the `Est` or `BlastX` plugins for exceptions).

1.5.1 Loading plugins

When EuGène starts, plugins are loaded and instanciated following parameters in the parameter file. The `Sensor.*.use` may activate or desactivate the corresponding sensor (which must be available in the PLUGINS directory). If the parameter value is set to `0` or `FALSE`, the plugin is not used. If the parameter value is set to `1`, then a single instance of the plugin is loaded. If the parameter is set to an integer value, then this number of instances of the plugin are created.

Below is the list of minimum plugins which are activated by default by the *Arabidopsis thaliana* version of EuGène.

```
Sensor.Transcript.use   1
Sensor.EuStop.use       1
Sensor.NStart.use       1
Sensor.IfElse.use       1 (with 2 splice site prediction plugins)
Sensor.MarkovIMM.use    1
Sensor.MarkovConst.use  1
```

Sensors are loaded and instanciated following an increasing order of priorities. The priority of a given type of plugin is defined by the value of the corresponding `Sensor.*` parameter. Here is an example of actual priorities:

```
Sensor.Transcript       1
Sensor.FrameShift       1
Sensor.IfElse           1
Sensor.EuStop           1
Sensor.NStart           1
Sensor.MarkovIMM        1
Sensor.Est              30
```

The `Sensor.Est` is loaded last because it has the highest priority. This is important since the sensor actually uses the information provided by other sensors (splice site prediction sensors) that then have to be loaded before.

Several instances of the same sensor can be loaded. Eg., if you are dealing with an organism that has a large GC% range, one may use several `Sensor.MarkovIMM`. Imagine you want to use one model for sequences who have a GC% below 50 and another for higher GC%. This can be achieved by instanciating 2 such sensors.

```
Sensor.MarkovIMM        2
```

When these sensors will be instanciated, they will look for specific parameters. The first instance will use the usual parameters or parameters followed by `[0]` for this plugin class, the second instance will use parameters followed by `[1]`.

```
MarkovIMM.matname[0]    lowGC.mat
MarkovIMM.minGC[0]      0
MarkovIMM.maxGC[0]      50
MarkovIMM.matname[1]    highGC.mat
MarkovIMM.minGC[1]      50
MarkovIMM.maxGC[1]      100
```

As the example show, it is equivalent to define the parameter `MarkovIMM.matname[0]` (or any parameter followed by `[0]` and the parameter `MarkovIMM.matname`.

1.5.2 `GFF3 input documentation`

Since version 3.4b, Eugene allows for Gff3-compliant input and output.

GFF3 format See `http://www.sequenceontology.org/gff3.shtml` for details. In a GF33 file, everything is line-based. The format of a line is:

`<seqid> <source> <type> <start> <end> <score> <strand> <phase><attributes>`

The attributes column is composed of tags, some tags have predefined meanings according to Gff3 specifications. These are the tags `ID, Target, Ontology_term`. For Eugene, we define additional specific attributes: `is_full_length, target_length, target_sequence, database, frame_hit, frame_hit, score_hit`.

A new parameter is needed in `eugene.par`: `Gff3.SoTerms cfg/sofa.obo`

It specifies the path relatively to EUGENEDIR of the file which contains all SOFA codes. Currently we use the version 1.2 of 25:07:2007. In order to create valid gff3 file, you have to use SOFA terms or codes.

The third column (type) of gff3 format must contain a term of SOFA, program accept the id, name and synonyms.

Example of SOFA definition term :

```
[Term]
id: SO:0000164
name: three_prime_splice_site
def: "The junction between the 3 prime end of an intron and
the following exon." [http://www.ucl.ac.uk/~ucbhjow/b241/glossary.html]
subset: SOFA
synonym: "3' splice site" RELATED []
synonym: "acceptor" RELATED []
synonym: "acceptor splice site" EXACT []
synonym: "splice acceptor site" EXACT []
is_a: SO:0000162 ! splice_site
```

The accepted types in the third columns are:

- `SO:0000164`
- `three_prime_splice_site`
- `acceptor`
- `acceptor splice site or acceptor_splice_site`
- `splice acceptor site or splice_acceptor_site`

Each plugin has its own extension, in gff3 mode you just have to add '.gff3' after the native file name. Example if plugin `SPred` is active with gff3 input format , it will expect a file named `file.SPred.gff3` (instead of `file.SPred` in native mode)

We now descrive each plugin, its behavior and parameters.

1.5.3 Signal plugins

1.5.3.1 Sensor.EuStop

Description This simple plugin predicts translation stops. It is able to deal with noisy sequences and will eg. predict a possible stop on `TGN`.

The sensor is activated by setting the value 1 for the parameter `Sensor.EuStop.use` in the parameter file.

The penalty payed for using a Stop is defined by the `EuStop.stopP*` parameter.

Here is an example of EuStop parameters definition.

```
EuStop.stopP*           4.155   # Stop penalty
Sensor.EuStop.use       1    # Use EuStop sensor
Sensor.EuStop           1       # Sensor priority
```

Input files format No input files needed.

Integration of information In the case of non degenerated sequences, all predictions using a Stop to end a terminal exon are given an extra `EuStop.stopP*` penalty. All predictions going through an in-phase Stop in an exonic state receive an infinite penalty.

Post analyse No post analyse.

Graph Every predicted Stop is ploted as a small vertical red bar in the corresponding phase on the exonics tracks.

1.5.3.2 Sensor.FrameShift

Description This plugin predicts possible frameshifts (either insertions or deletions) at each position of the sequence with a uniform cost. The parameters `FrameShift.Ins*` `FrameShift.Del*` give the corresponding penalties.

The sensor is activated by either:

- the `-f` argument .
- the value 1 for the parameter `Sensor.FrameShift.use` in the parameter file.

Here is an example of FrameShift parameters definition.

```
FrameShift.Ins*        1e999.0
FrameShift.Del*        1e999.0
Sensor.FrameShift.use  1     # Use FrameShift sensor
Sensor.FrameShift      1        # Sensor priority
```

Input files format No input files needed.

Integration of information All predictions that use a frameshift (going from one coding phase to another coding phase) are given an extra `FrameShift.Ins*` or `FrameShift.Del*` penalty according to the phase change.

Post analyse No post analyse.

Graph Every predicted frameshift is plotted as a vertical red line that connect the exonic prediction blocks in the 2 corresponding phase.

1.5.3.3 `Sensor.GSplicer`

Description The GSplicer sensor injects possible splice sites as predicted by the GeneSplicer program. The sensor is activated by the value 1 for the parameter `Sensor.GSplicer.use` in the parameter file. Here is an example of GSplicer parameters definition :

```
GSplicer.coefAcc*    0.8300      #
GSplicer.penAcc*     7.7000      # GSplicer parameters (rescaling)
GSplicer.coefDon*    1.3153      # See the Integration of
GSplicer.penDon*     10.1600     # information section.
Sensor.GSplicer.use  1        # Use GSplicer sensor
Sensor.GSplicer      1           # Sensor priority
```

Native input files format The plugin reads the prediction of the program from one file whose name is derived from the sequence name by adding the `.Gsplicer` suffix. This file describes the predicted splice sites for the forward and reverse strand.

The files `.Gsplicer` describe the predicted splice sites sorted by position on the sequence (as given by GeneSplicer). The format of a line is : `<End5> <End3> <Score> <confidence> <splice_site_type>`

Here is an extract from `SYNO_ARATH.fasta.Gsplicer` :

```
422 423 17.275659 High donor
512 513 15.534963 High acceptor
583 584 9.516534 Medium donor
697 698 6.432014 Medium acceptor
745 746 2.028095 Medium donor
810 811 9.255683 Medium donor
896 897 9.772425 Medium acceptor
1019 1020 10.580889 Medium donor
1105 1106 5.318046 Medium acceptor
1177 1178 7.345249 Medium acceptor
1203 1204 3.989773 Medium donor
1269 1270 15.249301 High acceptor
[...]
```

These files can be obtained by launching the GeneSplicer software available at:

- web site : `http://www.tigr.org/tdb/GeneSplicer/index.shtml`.
- ftp serveur : `ftp://ftp.tigr.org/pub/software/GeneSplicer`.

GeneSplicer is launched with the command:

```
genesplicer SEQ_FASTA GENOME_TRAINING_DIRECTORY [options] > SEQ_FASTA.Gsplicer
```

where options are:

- $-a\ t$: Choose t as a threshold for the acceptor sites
- $-d\ t$: Choose t as a threshold for the donor sites
- $-e\ n$: The maximum acceptor score within n bp is chosen
- $-i\ n$: The maximum donor score within n bp is chosen

Gff3 input file format The gff3 input mode is activated by setting the value `GFF3` for the parameter `Gsplicer.format` in the parameter file. The plugin reads the predictions of the program from one file which name is derived from the sequence name by adding the `.Gsplicer.gff3` extension.

Accepted features (third column):

- SO:0000164 or splice_acceptor_site
- SO:0000163 or splice_donor_site

If the feature used isn't one of those, the line will be rejected. The expected coordinates must correspond to the AG/GT nucleotides (this is checked). Here an extract of `seq14ac002535g4g5.tfa.Gsplicer.gff3`

```
seq14       GSplicer      SO:0000164      210       211       9.013469      -       .       ID=SO:0000164:seq14.1
seq14       GSplicer      SO:0000163      244       245       3.350242      -       .       ID=SO:0000163:seq14.1
seq14       GSplicer      SO:0000163      307       308       4.115466      -       .       ID=SO:0000163:seq14.2
```

Filtering input information No filter.

Integration of information The procedure consists in weigthing the graph used by EUGÈNE. For each predicted site the edge corresponding to the good transition is weighted by: $(s_i * coef) - pen$. Where s_i is the score given by GeneSplicer, $coef$ and pen are given in the parameter file.

A set of 8 vectors is used. The vectors are:

- $vPosAccF$ the forward acceptor predicted positions
- $vValAccF$ the forward acceptor score at position $vPosAccF$
- $vPosAccR$ the reverse acceptor predicted positions
- $vValAccR$ the reverse acceptor score at position $vPosAccR$
- $vPosDonF$ the forward donor predicted positions
- $vValDonF$ the forward donor score at position $vPosDonF$
- $vPosDonR$ the reverse donor predicted positions
- $vValDonR$ the reverse donor score at position $vPosDonR$

For each position if one of the 4 "position vectors" contains the query position:

- for forward and reverse acceptor sites : $(vValAcc*coefAcc)-penAcc$ is added to the corresponding transition (intron track to exon track according to the phase)
- for forward and reverse donor sites : $(vValDon*coefDon)-penDon$ is added to the corresponding transition (exon track to intron track according to the phase)

Post analyse No post analyse.

Graph Predicted splice sites are visible on the intronic tracks as green (donor) and magenta (acceptor) vertical lines whose length indicates the site score.

1.5.3.4 `Sensor.NG2`

Description This plugin injects possible splice sites as predicted by the NetGene2 program.

The sensor is activated by setting the value 1 for the parameter `Sensor.NG2.use` in the parameter file. The score for acceptor and donor prediction is rescaled by the parameters `NG2.accP*` and `NG2.accB*` for acceptors and `NG2.donP*` and `NG2.donB*` for donors (see below).

Here is an example of NG2 parameters definition.

```
NG2.accP*        0.903
NG2.accB*        5.585
NG2.donP*        0.980
NG2.donB*        27.670
Sensor.NG2.use   1
Sensor.NG2       1      # Sensor priority
```

Native input files format The plugin reads the prediction of the program from two files whose names are derived from the sequence name by adding the `.splices` and `.splicesR` suffixes (respectively prediction for the forward and reverse strand).

The files with `.splices` and `.splicesR` suffixes are obtained by running NetGene2 which can be obtained at `http://www.cbs.dtu.dk/services/NetGene2/` and using the detailed output of the software.

Here is an extract from `SYNO_ARATH.fasta.splices`:

```
    396 C  0      0      0.903 2  0.835  0.862  0      0       -     -
    397 T  0      0      0.858 3  0.828  0.869  0      0       -     -
    398 C  0      0      0.873 1  0.822  0.876  0      0       -     -
    399 A  0      0      0.826 2  0.816  0.882  0      0       -     -
    400 G  0      0      0.869 3  0.809  0.889  0      0     -2.337 359
    401 A  0      0      0.862 1  0.803  0.896  0      0       -     -
    402 G  0      0      0.794 2  0.798  0.901  0      0     -2.337 359
    403 C  0      0      0.809 3  0.792  0.907  0      0       -     -
    404 A  0      0      0.833 1  0.786  0.914  0      0       -     -
    405 G  0      0      0.823 2  0.780  0.920  0      0     -2.337 359
    406 T  0      0      0.845 3  0.774  0.926  0      0       -     -
    407 G  0      0      0.792 1  0.769  0.932  0      0       -     -
    408 T  0      0      0.745 2  0.764  0.936  0      0       -     -
    409 C  0      0      0.792 3  0.759  0.942  0      0       -     -
    410 A  0      0      0.802 1  0.753  0.948  0      0       -     -
    411 C  0      0      0.764 2  0.749  0.953  0      0       -     -
[...]
```

To run Netgene2, the following parameters are used (for *Arabidopsis thaliana*): `netgene2 -a -e -p -r -s at <Fasta sequence>`.

Gff3 input file format The gff3 input mode is activated by setting the value `GFF3` for the parameter `NG2.format` in the parameter file. The plugin reads the predictions of the program from one file which name is derived from the sequence name by adding the `.splices.gff3` extension.

Accepted features (third column):

- SO:0000164 or splice_acceptor_site
- SO:0000163 or splice_donor_site

If the feature used isn't one of those, the line will be rejected. The expected coordinates must match the AG/GT nucleotides. Here an extract of `seq14ac002535g4g5.tfa.splices.gff3`:

```
seq14       NG2      SO:0000163      18       19       0.586      +       .       ID=SO:0000163:seq14.1;
seq14       NG2      SO:0000163      27       28       0.614      +       .       ID=SO:0000163:seq14.2;
seq14       NG2      SO:0000164      57       58       0.017      +       .       ID=SO:0000164:seq14.1;
```

Filtering input information No filtering.

Integration of information The integrated score for donor/acceptor prediction is read (columns 9 and 10). If it is not available (extremities of the sequence) then the non integrated score is used (columns 3 and 4).

The score read s is rescaled using the `NG2.accP*` (*P*) and `NG2.accB*` (*B*) parameters for acceptors and `NG2.donP*` (*P*) and `NG2.donB*` (*B*) parameters for donors as follows:

$$s' = B * s^P$$

All predictions that use a predicted splice site receive a $\log(s')$ penalty while those that go through a predicted splice site while they could have used it receive a $\log(1 - s')$ penalty.

Post analyse No post analyse.

Graph Predicted splice sites are visible on the intronic tracks as green (donor) and magenta (acceptor) vertical lines whose length indicates the site score.

1.5.3.5 `Sensor.NStart`

Description This plugin injects possible translation starts as predicted by the NetStart program.

The sensor is activated by setting the value `1` for the parameter `Sensor.NStart.use` in the parameter file. The score for acceptor and donor prediction is rescaled by the parameters `NStart.startP*` and `NStart.startB*` (see below).

Here is an example of NetStart parameters definition.

```
NStart.startP*      0.052
NStart.startB*      0.308
Sensor.NStart.use   1        # Use NStart sensor
Sensor.NStart       1        # Sensor priority
```

Native input files format The plugin reads the prediction of the program from two files whose names are derived from the sequence name by adding the `.starts` and `.startsR` suffixes (respectively prediction for the forward and reverse strand).

The files with `.starts` and `.startsR` suffix are obtained by running NetStart which can be obtained at `http://www.cbs.dtu.dk/services/NetStart/` and using the detailed output of the software.

Here is an extract from `SYNO_ARATH.fasta.starts`:

```
  1089    0.256     -
  1146    0.214     -
  1251    0.618     Yes
  1299    0.197     -
  1474    0.526     Yes
  1535    0.112     -
  1559    0.490     -
  1638    0.401     -
  1674    0.569     Yes
  1678    0.147     -
  1740    0.299     -
  1752    0.187     -
[...]
```

To run NetStart, the following parameters are used (for *Arabidopsis thaliana*): `netstart -at <Fasta sequence>`.

Gff3 input file format The gff3 input mode is activated by setting the value `GFF3` for the parameter `NStart.format` in the parameter file. Then, the plugin reads the predictions of the program from one file which name is derived from the sequence name by adding the `.nstart.gff3` extension.

Accepted features (third column):

- SO:0000318 or start_codon

If the feature used isn't one of those, the line will be rejected. The expected coordinates must correspond to the first nucleotide of the start codon.

Here an extract of `seq14ac002535g4g5.tfa.starts.gff3`:

```
seq14     NStart     SO:0000318     64     64     0.299     +     .     ID=SO:0000318:seq14.0;
seq14     NStart     SO:0000318     74     74     0.249     +     .     ID=SO:0000318:seq14.1;
```

Filtering input information No filtering.

Integration of information The integrated score for start prediction is read (column 2). The score read s is rescaled using the `NStart.startP*` (*P*) and `NStart.startB*` (*B*) as follows:

$$s' = e^{-P} * s^B$$

All predictions that use a predicted start receive a $\log(s')$ penalty while those that go through a predicted start while they could have used it receive a $\log(1 - s')$ penalty.

Post analyse No post analyse.

Graph Predicted starts are visible on exonix tracks as blue vertical lines whose length indicates the site score.

1.5.3.6 `Sensor.PatConst`

Description This plugin predicts signals at each occurence of a pattern on the sequence. The corresponding uniform costs for using or rejecting a signal can be set using the `PatConst.patP*[i]`/ and `PatConst.patPNo*[i]` parameters.

The sensor is activated by setting the value 1 (one instance of the plugin) or an integer (i instance) for the parameter `Sensor.PatConst.use` in the parameter file.

Here is an example of PatConst parameters definition (2 instances) :

```
PatConst.type[0]        donor    # Possible types : start insertion deletion
PatConst.pat[0]         GC       #  transstart transstop stop acceptor donor
PatConst.newStatePos[0] 1        # Position of the new state in the pattern
PatConst.patP*[0]       -25
PatConst.patPNo*[0]     0
#
PatConst.type[1]        acceptor
PatConst.pat[1]         AG
PatConst.newStatePos[1] 3
PatConst.patP*[1]       -40
PatConst.patPNo*[1]     0
#
Sensor.PatConst.use     2
Sensor.PatConst         1        # Sensor priority
```

Input files format No file input.

Integration of information All predictions that use a predicted signal receive the corresponding `PatConst-.patP*[i]` penalty while those that go through a predicted splice site while they could have used it receive a `PatConst.patPNo*[i]` penalty.

Post analyse No post analyse.

Graph No plot.

1.5.3.7 `Sensor.PepSignal`

Description This plugin injects possible translation starts as predicted by the Predotar program (http://genoplante-info.infobiogen.fr/predotar/) that looks for peptide adressing sequences after every occurrence of an ATG.

The sensor is activated by setting the value `1` for the parameter `Sensor.PepSignal.use` in the parameter file. The score for start prediction is rescaled by the parameters `PepSignal.startP*` and `PepSignal.startB*` (see below).

Here is an example of PepSignal parameters definition.

```
PepSignal.startP*       0.9
PepSignal.startB*       0.1
Sensor.PepSignal.use    1       # Use PepSignal sensor
Sensor.PepSignal        10      # Sensor priority
```

Native input files format The plugin reads the predictions (both in forward and reverse strand) of the program from a file whose name is derived from the sequence name by adding the `.psignal` suffix.

The file `.psignal` describes the predicted start sites sorted by position on the sequence (as given by Predotar). The format of a line is : `<position> <strand> <score> <comment>`

Here is an example used for test:

```
175 start_rev 0.024083 test
188 start 0.000151 test
195 start_rev 0.010081 test
261 start 0.001628 test
270 start 0.000026 test
[...]
```

This file can be obtained by launching the Predotar software available at
`http://genoplante-info.infobiogen.fr/predotar/predotar.html`

Gff3 input file format The gff3 input mode is activated by setting the value `GFF3` for the parameter `PepSignal.format` in the parameter file. The plugin reads the predictions of the program from one file which name is derived from the sequence name by adding the `.psignal.gff3` extension.

Accepted features (third column):

- SO:0000318 or start_codon

If the feature used isn't one of these, the line will be rejected. The expected coordinates must match with the first nucleotide of a start codon (ATG).

Here an extract of `seq14ac002535g4g5.tfa.psignal.gff3`:

```
seq14       PepSignal       SO:0000318      175     175     0.024083        -       .       ID=SO:0000318:seq14.0;
seq14       PepSignal       SO:0000318      188     188     0.000151        +       .       ID=SO:0000318:seq14.1;
```

Filtering input information No filtering.

Integration of information The score (column 3) read s is rescaled using the `PepSignal.startP*` (*P*) and `PepSignal.startB*` (*B*) as follows:

$$s' = e^{-P}.s^{B}$$

All predictions that use a predicted start receive a $\log(s')$ penalty while those that go through a predicted start while they could have used it receive a $\log(1 - s')$ penalty.

Post analyse No post analyse.

Graph Predicted starts are visible on exonix tracks as blue vertical lines whose length indicates the site score.

1.5.3.8 Sensor.RibosomalFrameShift

Description This plugins simulates ribosomal frameshifts allowing alternative translation of a mRNA sequence by changing the open reading frame. At each occurence of the pattern `RibosomalFrameShift-.pat[i]` in the sequence, the plugin predicts a frameshift signal at the position `RibosomalFrameShift.newStatePos[i]` in the pattern. The parameter `RibosomalFrameShift.type[i]` represents the type of frameshift and its position according to the open reading frame. A -1 frameshift can be seen as the deletion of the nucleotide before the position `RibosomalFrameShift.newStatePos[i]`, while a +1 frameshift as the insertion of a nucleotide to the position `RibosomalFrameShift.newStatePos[i]`.

Three values allow to represent a -1 frameshift in specific open reading frames:

- `deletion1` : deletion of the first nucleotide of the codon
- `deletion2` : deletion of the second nucleotide of the codon
- `deletion3` : deletion of the third nucleotide of the codon

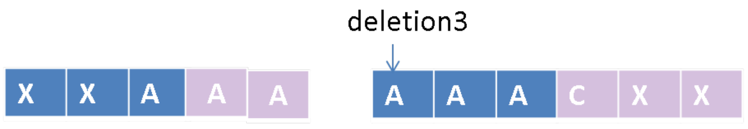
Three values allow to represent a +1 frameshift in specific open reading frames:

- `insertion1` : insertion of a nucleotide before the first nucleotide of the codon
- `insertion2` : insertion of a nucleotide before the second nucleotide of the codon
- `insertion3` : insertion of a nucleotide before the third nucleotide of the codon

Set `RibosomalFrameShift.requiredEstSupport[i]` to 1 to only predict ribosomal frameshift if there is an EST support.

The corresponding uniform costs for using or rejecting a signal can be set using the `RibosomalFrame-Shift.patP*[i]` and `RibosomalFrameShift.patPNo*[i]` parameters.

Here is an example of parameters to catch the -1 frameshift A_AA.A_AA.C (underscores separate codons in the initial frame and dots separate codons in the new -1 frame):



```
RibosomalFrameShift.type[0] deletion3
RibosomalFrameShift.pat[0]  AAAAAAC
RibosomalFrameShift.newStatePos[0] 4   # Position of the frameshift in the pattern
RibosomalFrameShift.patP*[0] -25
RibosomalFrameShift.patPNo*[0] 0
RibosomalFrameShift.requiredEstSupport[0] 0 # 1 if an EST support is required
#
Sensor.RibosomalFrameShift.use      1
Sensor.RibosomalFrameShift          1        # Sensor priority
```

The sensor is activated by setting the value 1 (one instance of the plugin) or an integer (i instance) for the parameter `Sensor.RibosomalFrameShift.use` in the parameter file.

Input files format No file input.

Integration of information All predictions that use a predicted frameshift receive the corresponding `RibosomalFrameShift.patP*[i]` penalty while those that go through a predicted frameshift while they could have used it receive a `RibosomalFrameShift.patPNo*[i]` penalty.

Post analyse No post analyse.

Graph No plot.

1.5.3.9 `Sensor.SMachine`

Description This plugin injects possible start and splice sites as predicted by the SpliceMachine program. For more detail, see the publication Degroeve, S., Saeys, Y., De Baets, B., Rouzé, P., Van de Peer, Y. (2004) Predicting splice sites from high-dimensional local context representations Bioinformatics.

There are two possible outputs for this program, the direct raw output directly outputs SVM values (positive or negative real numbers with arbitrary magnitude) or a rescaled output (fitting to a sigmoid) with a positive ouput between 0 and 1 (with a probabilistic interpretation). The two different outputs may lead to different prediction performances in EuGène.

The sensor is activated by setting the value 1 for the parameter `Sensor.SMachine.use` in the parameter file. The score for start, acceptor and donor prediction is rescaled by the parameters `SMachine.startP*` and `SMachine.startB*` for starts, `SMachine.accP*` and `SMachine.accB*` for acceptors and `SMachine.donP*` and `SMachine.donB*` for donors (see below).

The parameter `SMachine.isScaled` indicates how the scores of SpliceMachine are integrated in EuGène (the details of the scaling used in each case is given below. Note that this is the second rescaling if the sigmoid fitting has been used in SpliceMachine). The parameter `SMachine.cmd` contains the command which is launch if the predictions files do not exist.

Here is an example of SMachine parameters definition.

```
SMachine.cmd            "splicemachine.pl "
SMachine.isScaled       1
SMachine.accP*          0.102032725565
SMachine.accB*          5.585
SMachine.donP*          0.020202707318
SMachine.donB*          27.670
SMachine.startP*        0.052
SMachine.startB*        0.308
Sensor.SMachine.use     1                 # Use SMachine sensor
Sensor.SMachine         10                # Sensor priority
```

Native input files format The plugin reads the predictions of the program from two files whose names are derived from the sequence name by adding the `.spliceMSt` and `.spliceMAD` suffixes (respectively prediction for the starts and splices sites)

The files with `.spliceMSt` and `.spliceMAD` suffixes are obtained by running SpliceMachine which can be obtained at
`http://bioinformatics.psb.ugent.be/webtools/splicemachine/`

Here is an extract from a `.spliceMSt` file:

```
175 start_rev 0.024083
188 start 0.000151
195 start_rev 0.010081
261 start 0.001628
270 start 0.000026
[...]
```

Here is an extract from a `.spliceMAD` file:

```
210 acceptor_rev 0.066414
245 donor_rev 0.001345
628 acceptor 0.066414
1309 donor 0.000039
[...]
```

Gff3 input file format The gff3 input mode is activated by setting the value `GFF3` for the parameter `SMachine.format` in the parameter file. The plugin reads the predictions of the program from one file which name is derived from the sequence name by adding the `.spliceM.gff3` extension.

Accepted features (third column):

- SO:0000318 or start_codon, the expected coordinates must correspond to the first nucleotide of the start codon.
- SO:0000164 or splice_acceptor_site, the expected coordinates must correspond to the AG/GT nucleotides.
- SO:0000163 or splice_donor_site, the expected coordinates must correspond to the AG/GT nucleotides.

If the feature used isn't one of those, the line will be rejected. The expected coordinates must match the AG/GT nucleotides (splices) or ATG (start). Here an extract of `seq14ac002535g4g5.tfa.spliceM.gff3`.

```
seq14        SMachine        start_codon        175        175        0.024083        -        .        ID=start_codon:seq14.1;
seq14        SMachine        start_codon        188        188        0.000151        +        .        ID=start_codon:seq14.2;
seq14        SMachine        acceptor_splice_site        210        211        0.066414        -        .        ID=acceptor_splice_site:seq14.1;
seq14        SMachine        donor_splice_site        244        245        0.001345        -        .        ID=donor_splice_site:seq14.1;
```

Filtering input information No filtering.

Integration of information The integrated score for start and donor/acceptor prediction is read (columns 3). The score read s is rescaled using the `SMachine.startP*` (*P*) and `SMachine.startB*` (*B*) parameters for starts, `SMachine.accP*` (*P*) and `SMachine.accB*` (*B*) parameters for acceptors and `SMachine.donP*` (*P*) and `SMachine.donB*` (*B*) parameters for donors.

If (`SMachine.isScaled` is set to `0`) then the rescaled score s' is:

$$s' = B * s - P$$

used when the signal is used. If the signal is not used, no penalty occurs.

If (`SMachine.isScaled` is set to `1`) then the rescaled score s' is:

$$s' = B\log(s) - P$$

when the signal is used, and $\log(1.0 - s^B * e^{-P})$ otherwise.

If (`SMachine.isScaled` is set to `2`) then the rescaled score s' is

$$s' = B\log(s) - P$$

when the signal is used, nothing otherwise.

Post analyse No post analyse.

Graph Predicted starts are visible on exonix tracks as blue vertical lines whose length indicates the site score. Predicted splice sites are visible on the intronic tracks as green (donor) and magenta (acceptor) vertical lines whose length indicates the site score.

1.5.3.10 `Sensor.SpliceWAM`

Description The goal of the SpliceWAM sensor is to detect splice sites and to give them a score reflecting the context accordance with given models. A score is attributed at each potential acceptor and donor AG/GT site according to Weight Array Method (see Zhang and Marr, *Comput Appl Biosci.* 1993 Oct;9(5):499-509), or Weighted Array Matrix models (Salzberg, *Comput Appl Biosci* 1997 Aug;13(4):365-76). A WAM describes a consensus motif of a functional signal, and is composed by one markovian model per each position of the motif. Here the motifs are defined by the AG/GT (assumed to be present in all splice sites) plus two flanking contexts (used by the WAM). Globally, the score of a motif is a function of the emission probabilities of this motif given a true positive model and a false positive model.

The sensor is activated by setting the parameter `Sensor.SpliceWAM.use` to 1. The user have to specify in the parameter file the base name (prefix) of the model files (`SpliceWAM.donmodelfilename` and `SpliceWAM.accmodelfilename`), the size of the context (`SpliceWAM.NbNtBeforeGT`, `SpliceWAM.NbNtAfterGT` and `SpliceWAM.NbNtBeforeAG, SpliceWAM.NbNtAfterAG`), the order of the markovian models `SpliceWAM.MarkovianOrder` (the same for each position of the motifs), and the scaling parameters `SpliceWAM.DonScaleCoef*`, `SpliceWAM.DonScalePenalty*`, `SpliceWAM.AccScaleCoef*` and `SpliceWAM.ScalePenalty*`.

Here is an example of SpliceWAM parameters definition.

```
SpliceWAM.MarkovianOrder        1
SpliceWAM.donmodelfilename      WAM/WAM.ARA.DON.L9
SpliceWAM.NbNtBeforeGT          3
SpliceWAM.NbNtAfterGT           4
SpliceWAM.DonScaleCoef*         2.9004
SpliceWAM.DonScalePenalty*      -7.5877
SpliceWAM.accmodelfilename      WAM/WAM.ARA.ACC.L7
SpliceWAM.NbNtBeforeAG          2
SpliceWAM.NbNtAfterAG           3
SpliceWAM.AccScaleCoef*         2.9004
SpliceWAM.AccScalePenalty*      -7.5877
Sensor.SpliceWAM.use            1                # Use SpliceWAM sensor
Sensor.SpliceWAM                1                   # Sensor priority
```

Input files format This SpliceWAM Sensor requires a true positive and a false positive model file per motif position and for each type so sites. These files have to be present in the path given by `SpliceWAM.donmodelfilename` and `SpliceWAM.donmodelfilename` from the plugins directory (see `EuGene.PluginsDir` parameter). These models can be generated using `WAMbuilder.cc` (see `eugene/src/SensorPlugins/0_SensorTk/GetData/README`). The file name of a model is a concatenation of the base name (prefix) specified in the parameter file, an extension (suffix) specified in the `WAM.h` file (`.TP.` for true positive and `.FP.` for false positive), and a number between 00 and 99 indexing the position in the motif (restricting thus the motif length to a maximum of 100 nt).

As an example, with the base name `WAM.ARA.DON.L9` (refering to *A.thaliana* models of 9nt-length donor motif), one can found these files:

```
WAM.ARA.DON.L9.FP.00
WAM.ARA.DON.L9.FP.01
...
WAM.ARA.DON.L9.FP.07
WAM.ARA.DON.L9.FP.08
WAM.ARA.DON.L9.TP.00
WAM.ARA.DON.L9.TP.01
...
WAM.ARA.DON.L9.TP.08
```

These files are in binary form, each containing the properties of a markovian model (see documentations of `WAMbuilder.cc` and `markov.cc`).

Filtering input information Each binary model file is verified when loaded, checking if 3 expected properties of its markovian model are verified: the order, the alphabet size, and the total number of possible words (these 3 values are automatically included during the models generation by `WAMbuilder.cc`). This test is done in the loading file method "`chargefichier`" in markov.cc.

Integration of information At each AG/GT occuurence in the genomic sequence, a score is assigned depending on the AG/GT flanking context. If there isn't enough context, e.g. in the sequence extremities, nothing is done. This score is provided by a scaled sum of likelihood ratio, computed as following.

Let be P_i^t the emission probability of the nucleotid at position i in the motif according to the True Positive model, and P_i^f the emission probability of the nucleotid given by the False Positive model. The score given by the WAM for the entire motif M of length L is:

$$S_M = \sum_{i=0}^{L} log\left(\frac{P_i^t}{P_i^f}\right)$$

This score is then scaled with the `SpliceWAM.DonScaleCoef*`/`SpliceWAM.AccScaleCoef*` and the `SpliceWAM.DonScalePenalty*`/`SpliceWAM.AccScalePenalty*` parameters, following this formula :

$$S_M.\texttt{SpliceWAMScaleCoef*} + \texttt{SpliceWAMScalePenalty*}$$

This rescaled score is finally integrated into the EUGÈNE graph on the intron/exon transition edges at the corresponding positions. The score applies only to the edge corresponding to the situation where the signal is used. The edge corresponding to the situation where the signal is not used is unchanged.

Post analyse No Post-Analyse.

Graph Vertical green/magenta lines (whose length is function of the score) are plotted on the intron track on the corresponding strand for each splice site occurrence whose score is higher than a defined threshold. This threshold is defined in the `SpliceWAM.cc` file as -`plotscoreincrease`.

1.5.3.11 `Sensor.SPred`

Description This plugin injects possible splice sites as predicted by the SplicePredictor program.

The sensor is activated by setting the value 1 for the parameter `Sensor.SPred.use` in the parameter file. The score for acceptor and donor prediction is rescaled by the parameters `SPred.accP*` and `SPred.accB*` for acceptors and `SPred.donP*` and `SPred.donB*` for donors (see below).

Here is an example of SPred parameters definition.

```
SPred.accP*        0.987
SPred.accB*        3.850
SPred.donP*        0.929
SPred.donB*        10.800
Sensor.SPred.use   1
Sensor.SPred       1       # Sensor priority
```

Native input files format The plugin reads the prediction of the program from two files whose names are derived from the sequence name by adding the `.spliceP` and `.splicePR` suffixes (respectively prediction for the forward and reverse strand).

The files with `.spliceP` and `.splicePR` suffix are obtained by running SplicePredictor which can be obtained at `http://bioinformatics.iastate.edu/cgi-bin/sp.cgi`.

Here is an extract from `SYNO_ARATH.fasta.spliceP`:

```
[...]
A     <- 568 tgacttcggatgcAGaa   0.006 0.000 0.000  3 (1 1 1) IAEEEDA-E-EEEDIAD
A     <- 571 cttcggatgcagaAGgg   0.001 0.000 0.000  3 (1 1 1) AEEEDAE-E-EEDIADI
D -->    573           aggGTatga 0.176 0.009 0.000  6 (2 3 1) EEEDAEE-E-EDIADII
A     <- 582 gaagggtatgatcAGgt   0.003 0.000 0.000  3 (1 1 1) EEDAEEE-E-DIADIII
D -----> 583           cagGTaatt 0.964 0.256 0.788 15 (5 5 5) EDAEEEE-D-IAEEEED
A     <- 658 tatgataatccttAGac   0.001 0.000 0.000  3 (1 1 1) DAEEEED-I-AEEEEDI
A   <--- 698 ttgggtggataatAGgt   0.273 0.049 0.266 10 (3 3 4) AEEEEDI-A-EEEEDII
D ->     699           tagGTagaa 0.006 0.000 0.000  3 (1 1 1) AEEEDIA-E-EEEDIII
A     <- 702 gtggataataggtAGaa   0.001 0.000 0.000  3 (1 1 1) IIADIAD-I-IIIIIAD
D ->     709           ctgGTtcga 0.005 0.000 0.000  3 (1 1 1) IADIADI-I-IIIIAED
A     <- 744 cagtatctgtacaAGgt   0.007 0.000 0.000  3 (1 1 1) ADIADII-I-IIIAEDI
D ->     745           aagGTacta 0.042 0.000 0.000  3 (1 1 1) DIADIII-I-IIAEDIA
A     <- 756 aaggtactattgtAGct   0.007 0.000 0.000  3 (1 1 1) IADIIII-I-IAEDIIA
A     <- 760 tactattgtagctAGcc   0.002 0.000 0.000  3 (1 1 1) ADIIIII-I-AEDIIAE
A     <- 764 attgtagctagccAGgg   0.025 0.000 0.023  4 (1 1 2) DIIIIII-A-EDIIAEE
D ->     792           aagGTggag 0.057 0.001 0.000  3 (1 1 1) IIIIIIA-E-DIIAEEE
[...]
```

Gff3 input file format The gff3 input mode is activated by setting the value `GFF3` for the parameter `SPred.format` in the parameter file. The plugin reads the predictions of the program from one file which name is derived from the sequence name by adding the `.spliceP.gff3` extension.

Accepted features (third column):

- SO:0000164 or splice_acceptor_site
- SO:0000163 or splice_donor_site

If the feature used isn't one of those, the line will be rejected. The expected coordinates must match the AG/GT nucleotides.

Here an extract of `seq14ac002535g4g5.tfa.spliceP.gff3`.

```
seq14   SPred   SO:0000164      922     923     0.002   +       .       ID=SO:0000164:seq14.16;
seq14   SPred   SO:0000163      1098    1099    0.137   +       .       ID=SO:0000163:seq14.8;
```

Filtering input information No filtering.

Integration of information One of the SplicePredictor scores s for a given position is rescaled using the $\log(\alpha.s^{\beta})$ function. The four parameters `SPred.accP*`, `SPred.accB*`, `SPred.donP*`, `SPred.donB*` indicates the values of these α and β parameters for acceptor sites and donor sites respectively. These parameters have been estimated on existing data.

Post analyse No post analyse.

Graph Predicted splice sites are visible on the intronic tracks as green (donor) and magenta (acceptor) vertical lines whose length indicates the site score.

1.5.3.12 `Sensor.StartWAM`

Description The goal of the StartWAM sensor is to detect the translation start codons and to give them a score reflecting the context accordance with given models. A score is attributed at each potential start codons (ATG), according to Weight Array Method (see Zhang and Marr, *Comput Appl Biosci.* 1993 Oct;9(5):499-509), or Weighted Array Matrix models (Salzberg, *Comput Appl Biosci* 1997 Aug;13(4):365-76). A WAM describes a consensus motif of a functional signal, and is composed by one markovian model per each position of the motif. Here the motif is defined by the ATG (present in all start codons) plus the two flanking contexts (used by the WAM). Globally, the score of a motif is a function of the emission probabilities of this motif given a true positive model and a false positive model.

The sensor is activated by setting the parameter `Sensor.StartWAM.use` to 1. The user have to specify in the parameter file the base name (prefix) of the model files (`StartWAM.modelfilename`), the size of the context (`StartWAM.NbNtBeforeATG, StartWAM.NbNtAfterATG`), the order of the markovian models `StartWAM.MarkovianOrder` (the same for each position of the motif), and the scaling parameters `StartWAM.ScaleCoef*, StartWAM.ScalePenalty*`.

Here is an example of StartWAM parameters definition.

```
StartWAM.modelfilename      WAM/WAM.ARA.START  # base name of the model files
StartWAM.NbNtBeforeATG      3                  # amount context
StartWAM.NbNtAfterATG       3                  # aval context
StartWAM.MarkovianOrder     1                  # order of the markovian models
StartWAM.ScaleCoef*         0.1594             # scaling parameter
StartWAM.ScalePenalty*      -3.1439            # scaling parameter
Sensor.StartWAM.use         1               # Use StartWAM sensor
Sensor.StartWAM             1                  # Sensor priority
```

Input files format This StartWAM sensor requires a true positive and a false positive model file per motif position. These files have to be present in the path given by `StartWAM.modelfilename` from the plugins directory (see `EuGene.PluginsDir` parameter). These models can be generated using `WAMbuilder.cc` (see `eugene/src/SensorPlugins/0_SensorTk/GetData/README`). The file name of a model is a concatenation of the base name (prefix) specified in the parameter file, an extension (suffix) specified in the `WAM.h` file (`.TP.` for true positive and `.FP.` for false positive), and a number between 00 and 99 indexing the position in the motif (restricting thus the motif length to a maximum of 100 nt).

As an example, with the base name `WAM.ARA.START9` (refering to *A.thaliana* models of 9nt-length start motif), one can found these files:

```
WAM.ARA.START9.FP.00
WAM.ARA.START9.FP.01
...
WAM.ARA.START9.FP.07
WAM.ARA.START9.FP.08
WAM.ARA.START9.TP.00
WAM.ARA.START9.TP.01
...
WAM.ARA.START9.TP.08
```

These files are in binary form, each containing the properties of a markovian model (see documentations of `WAMbuilder.cc` and `markov.cc`).

Filtering input information Each binary model file is verified when loaded, checking if 3 expected properties of its markovian model are verified: the order, the alphabet size, and the total number of possible words (these 3 values are automatically included during the models generation by `WAMbuilder.cc`). This test is done in the loading file method "`chargefichier`" in markov.cc.

Integration of information At each ATG of the genomic sequence a score is assigned depending on the ATG flanking context. If there isn't enough context, e.g. in the sequence extremities, nothing is done. This score is provided by a scaled sum of likelihood ratio, computed as following.

Let be P_i^t the emission probability of the nucleotid at position i in the motif according to the True Positive model, and P_i^f the emission probability of the nucleotid given by the False Positive model. The score given by the WAM for the entire motif M of length L is:

$$S_M = \sum_{i=0}^{L} log\left(\frac{P_i^t}{P_i^f}\right)$$

This score is then scaled with the `StartWAM.ScaleCoef*` and the `StartWAM.ScalePenalty*` parameters, following this formula :

$$S_M.\texttt{StartWAMScaleCoef}\star + \texttt{StartWAMScalePenalty}\star$$

This rescaled score is finally integrated into the EuGène graph on the UTR5 $\rightarrow$ EXON transition edge just before the considered ATG. The score applies only to the edge corresponding to the situation where the signal is used. The edge corresponding to the situation where the signal is not used is unchanged.

Post analyse No Post-Analyse.

Graph Vertical blue lines (whose length is function of the score) are plotted on the corresponding frame for each start codon which score is higher than a defined treshold. This threshold is defined in the `StartWAM.cc` file as -`PlotScoreIncrease`.

1.5.3.13 `Sensor.Transcript`

Description This simple plugin predicts a possible transcription start and stop, and a possible transcription start and stop for non protein coding RNA, at every position, all with the same uniform cost. These costs are respectively set by the `Transcript.Start*`, `Transcript.Stop*`, `Transcript.StartNpc*` and `Transcript.StopNpc*` parameters.

The sensor is activated by setting the value 1 for the parameter `Sensor.Transcript.use` in the parameter file.

Here is an example of Transcript parameters definition.

```
Transcript.Start*       4.155
Transcript.Stop*        4.155
Transcript.StartNpc*    30
Transcript.StopNpc*     30
Sensor.Transcript.use   1   # Use Transcript sensor
Sensor.Transcript       1      # Sensor priority
```

Input files format No input file needed.

Integration of information All predictions that go through a transcription start (resp. stop) are penalized by the corresponding `Transcript.Start*` (resp. `Transcript.Stop*`) parameter value. All predictions that go through a transcription start (resp. stop) of a non protein coding RNA are penalized by the corresponding `Transcript.StartNpc*` (resp. `Transcript.StopNpc*`) parameter value.

Post analyse No post analyse.

Graph No plotting.

1.5.3.14 `Sensor.ProStart`

Description This plugin is dedicated to inject possible translation starts in prokaryote sequences.

This sensor is activated by setting the value 1 for the parameter `Sensor.ProStart.use` in the parameter file.

`ProStart.stackFile` and `ProStart.loopFile` contain the energetic parameters for computing the RBS (Shine-Delgarno) hybridization energy. (The file path is relative to EUGENEDIR/models). It is taken and adapted from Turners website. EuGène uses a simplified RNA binding model which ignores stabilizing loops (eg. tetraloops). The algorithm is essentially a free end-gap global alignement algorithm that takes into account bulges, loops and helices.

`ProStart.alpha*` and `ProStart.beta*`: Start parameters α and β. These parameters are used to control how the RBS quality affect the probability of use of a given START codon. If E is the hybridization energy of the RBS, the following probability is used:

$$\frac{\alpha}{1+\beta\cdot\exp(E/RT)}$$

So, α gives a basic probability for a START to be used. For β, the higher it is, the stronger the influence of the RBS quality on the use of the corresponding START. START probability is also affected by their type: `ATG` is the preferred START codon, then `GTG` and `TTG`.

Here an example of ProStart parameters definition:

```
ProStart.matchlen 14
ProStart.matchoffset 3
ProStart.RBSPattern attcctcca # E coli (16S genetics/ Steitz J.A.)
ProStart.alpha* 0.4
ProStart.beta* 6.0
ProStart.stackFile stack.dat
ProStart.loopFile loop.dat

Sensor.ProStart.use 1
Sensor.ProStart 10
```

Input files format No input files needed

Integration of information If E is the hybridization energy of the RBS, the following probability is used:

$$p=\frac{\alpha}{1+\beta\cdot\exp(E/RT)}$$

All predictions that use a predicted start receive a $\log(p)$ penalty while those that go through a predicted start while they could have used it receive a $\log(1-p)$ penalty.

Post analyse No post analyse.

Graph Predicted starts are visible on exonix tracks as blue vertical lines whose length indicates the site score.

1.5.4 Content plugins

1.5.4.1 Sensor.BlastX

Description The BlastX sensor allows to exploit similarities with homologous proteins. The similarities influence exon and intron detection. Similarities from several databases can be exploited. Usually 3 databases are used: SwissProt, PIR and TrEMBL.

A label i (that could vary from 0 to 9) is assigned at each considered database. Files describing a collection of similarities with a sequence have an extension .blast<i> (`.blast0,...,.blast9`).

The user has to specify the list of labels to consider, the confidence accorded to each, the minimum length of an intron and a number of amino acids involved in intron incitation. The sensor is activated by either:

- the `-b` argument that allows to specify the labels to consider, for example `-b092` to use the levels 0, 9, 2 (files `.blast0, .blast9, .blast2`),
- the value 1 for the parameter `Sensor.BlastX.use` in the parameter file and the labels to consider in the `.BlastX.levels` parameter.

The confidence in analyzes have to be specified in the parameter file giving values to the parameters `BlastX.level`<i>*. The minimum length of an intron is defined in the `BlastX.minIn` parameter. A number of amino acids defined in the `BlastX.blastxM*` parameter that allows to define if 2 similarities are near (see the paragraph Integration of information). Finally the `BlastX.postProcess` parameter (when set to 1) allows to request to analyse how BlastX information are integrated in the final prediction.

Here is an example of BlastX parameters definition.

```
BlastX.postProcess 1     # analyse prediction accorded to BlastX information
BlastX.levels      012      # use levels 0, 1, and 2
BlastX.activegaps      0    # make gap active on level 0
BlastX.level0*     0.2      # confidence in the level 0
BlastX.level1*     0.0      # confidence in the level 1
BlastX.level2*     0.0      # confidence in the level 2
BlastX.blastxM*    10       # nb of amino acids implicated in intron incitation
BlastX.minIn       50       # minimum length of intron
Sensor.BlastX.use  1     # Use BlastX sensor
Sensor.BlastX      1        # Sensor priority
```

Native input files format The files `.blast<i>` describe a collection of similarities sorted by protein and by position on the sequence. One similarity S is described per line.

The format of a line is:

<b^S> <e^S> <s^S> <v^S> <p^S> `<protein name>` <bp^S> <ep^S>

where:

- b^S and e^S are the begin and the end of the similarity S on the sequence,

- s^S is the score of the similarity S,
- v^S is the e-value given by BlastX and ignored by EUGÈNE,
- p^S is the phase: +1, +2, +3, -1, -2, -3,
- bp^S and $ep^S$are the begin and the end of the similarity S on the protein.

Here is an extract from `SYNO_ARATH.fasta.blast0`:

```
2820 2861 36 3e-08 +3  sp_O07683_SYD_HALSA; 335 348
2972 3088 41 3e-08 +2  sp_O07683_SYD_HALSA; 359 397
3185 3298 113 3e-08 +2  sp_O07683_SYD_HALSA; 398 435
353 418 45 2e-13 +2  sp_O24822_SYD_HALVO; 13 34
1850 1915 67 2e-13 +2  sp_O24822_SYD_HALVO; 202 223
2775 2858 72 2e-13 +3  sp_O24822_SYD_HALVO; 318 345
3191 3280 104 2e-13 +2  sp_O24822_SYD_HALVO; 397 426
353 418 51 7e-12 +2  sp_O26328_SYD_METTH; 21 42
1271 1414 70 7e-12 +2  sp_O26328_SYD_METTH; 141 188
1850 1954 62 7e-12 +2  sp_O26328_SYD_METTH; 210 244
3191 3280 93 7e-12 +2  sp_O26328_SYD_METTH; 401 430
```

These files can be obtained directly from the output BlastX files by parsing them with the `blast_parser.pl` script. The BlastX is launched with the command:

```
blastall -p blastx -d DATABASE_MULTIFASTA_PROTEIC_FILE -g F -F T -b
500000 -v 500000 -e 1e-6 -i QUERY_GENOMIC_SEQUENCE_FASTA >
TEMPORY_BLAST_RESULT_FILE
```

and the final `.blast`$< i >$ files are obtained with:

```
blast_parser.pl TEMPORY_BLAST_RESULT_FILE | sort -n -k 1,1 | sort -s
-k 6,6 > QUERY_GENOMIC_SEQUENCE_FASTA.blast0
```

For more explanation, see the README file in the directory `eugene/src/SensorPlugins/BlastX/GetData`.

Gff3 input file format The gff3 input mode is activated by setting the value `GFF3` for the parameter `BlastX.format` in the parameter file. The plugin reads a collection of similarities from files named with the sequence name and an extension .blast<i>.gff3 (`.blast0.gff3, ..., .blast9.gff3`).

Each similarity is described by a GFF3 line as follows

`<sequence name> <program> <sofa feature>` <b^S> <e^S> <v^S> `<strand> <.> ID=...;Target=`
`name>` <bp^S> <ep^S>`;frame_hit=`<p^S>`;score_hit=`<s^S>

Gff3 attributes specifications:
Required attributes:

- ID
- Target

Optional attributes:

- frame_hit represent the blast frame. This information can be omitted, but if it's specified, eugene will check it is consistent with the similarity positions and reject the information otherwise.

- score_hit : normalized score.

Here an extract of : seq14ac002535g4g5.tfa.blast0.gff3

```
seq14        blastx0        match        2361        4506        .        +        .        ID=blastx0:seq14.1;Dbxref=blastx0:sp_O84903_GALE_LACCA
seq14        blastx0        match_part        2361        2483        2e-07        +        .        ID=blastx0:seq14.1.1;Dbxref=blastx0:sp_O84903_GALE_LACCA;Target=sp_O
seq14        blastx0        match_part        2792        2857        2e-07        +        .        ID=blastx0:seq14.1.2;Dbxref=blastx0:sp_O84903_GALE_LACCA;Target=sp_O
seq14        blastx0        match_part        4432        4506        2e-07        +        .        ID=blastx0:seq14.1.3;Dbxref=blastx0:sp_O84903_GALE_LACCA;Target=sp_O
```

Filtering input information Similarities with a length higher than 15,000 nucleodites are rejected. A message "Similarity of extreme length rejected" is printed to alert the user.

Integration of information The procedure consists first, in computing information at the nucleotide level and second, in weigthing the graph used by EUGÈNE.

A/ Computing information at the nucleotide level

A-1/ Extracting information

Each similarity S is considered, one after the other. A set of 3 variables is computed for nucleotide in position i. The variables are:

- s_i the score of the nucleotide at position i
- c_i the confidence in s_i,
- p_i the phase of s_i : +1, +2, +3, -1, -2, -3 for exon and 0 for intron,

Let l^S be the length of the similarity in nucleotide.

$$l^S = (ep^S - bp^S - 1) * 3$$

<u>Valuation for exon position</u>

- from $i = b^S$ to $i = e^S$
  - $s_i = s^S / l^S$
  - $c_i = c^S$
  - $p_i = p^S$

<u>Valuation for intron position</u>

An intron is consider as more likely when there is sufficient gap between 2 HSP on the genomic sequence and the corresponding positions on the same protein are close together:

- on the protein side, two HSP of the same protein appear on the same strand with a maximum gap or overlap bteween the HSPs of `BlastX.blastxM*` amino acids.
- on the genomic side, the length of the gap (in nucleotide) between the two HSPs is larger than `BlastX.minIn`.

Considering a gap S between 2 HSPs in these conditions, intron predictions are favored on a small region of length `BlastX.minIn`/2 on both sides of the gap (inside the gap).

- from $i = b^S -$ BlastX.minIn$/2$ to $i = b^S$

- from $i = e^S$ to $i = e^S+$ BlastX.minIn$/2$
- the following values are given at each position:
  - $s_i = s^S/l^S$
  - $c_i = c^S$
  - $p_i = 0$

If furthermore, the `activegaps` parameter is set for a level, then for all the bases in the gap S which have not already been considered in the step above, the intergenic, UTR and UTR introns states are also penalized. The penality used is the minimum of the weights used for the left and right HSP around the gap S.

A-2/ Combining extracted information

When all the similarities have been handled, if a position has several set of variables, the set with the highest confidence is kept. In case of egal confidence, the set with the higher score is kept.

B/ Weighting the graph

For each i with a set of variables:

- if p_i codes for exon then $s_i.c_i$ is added to the content score of nucleotide i in the corresponding exon phase (a track between 0 and 5),
- if p_i codes for intron then $s_i.c_i$ is added to the intron score of nucleotide i (tracks 6 and 7),

Note: in fact, instead of rewarding the correct track (like described here), all the tracks except the according one(s) are penalized, with a penalty equal to $-|s_i.c_i|$.

Post analyse The correspondance between BlastX information and prediction is analyzed if the `-B` flasg is provided or if the `BlastX.PostProcess` parameter is set to `1`.

For each predicted CDS, from the start codon to the stop, the percentage of nucleotides supported by a proteic similarity is displayed.

Graph Grey horizontal lines are ploted on the exon tracks for only the 3 first levels to consider (dark grey for the first, grey for the second, and light grey for the third).

1.5.4.2 `Sensor.Est`

Description This sensor is intended to take into account information from aligned transcribed sequences, both complete cDNA and EST. The existence of a hit (resp. gap) in the spliced alignment will influence intergenic, exonic and intronic state costs by penalizing states that are incompatible with the alignment. The spliced alignments must be performed beforehand using a spliced aligner such as `sim4` or `spidey`. The output of these aligners must be converted in the adequate format (see below). To also take into account information from ncRNA hits, set `Est.mRNAOnly` to 0.

The sensor is activated by either:

- the `-d` argument .
- a value higher than 0 for the parameter `Sensor.Est.use` in the parameter file.

The behavior of the plugin is controlled by the following parameters:

- `Est.estP*` indicates the penalty for violating a transcribed evidence.
- `Est.CdsBoost*` is a bonus used to enhance the score of the CDS regions which match with a transcribed evidence. Use to promote CDS regions instead of UTR regions.
- `Est.SpliceBoost*` is a bonus used to enhance the score of predicted splice sites which appear at a hit/gap border of aligned spliced ESTs.
- `Est.SpliceNonCanP` is the penalty affected if a non canonical splice site is detected on the border of transcribed evidence. Note that non canonical splice sites are researched only if `EuGene.NonCanAcc` and/or `EuGene.NonCanDon` are defined.
- `Est.SpliceStartP` is the penalty for all predictions that go through a spliced start at the frontier of the first and the second match region in a genomic spliced alignment. Note that the minimum length of the first coding exon has to be 1 nt to allow the detection of spliced start. (check the value in `EuGene.InitExDist` file)
- `Est.estM` gives the amount of "fuzzyness" allowed in interpreting a hit/gap border. The nucleotides which are less than `Est.estM` nucleotides away from this border are considered as neither in a hit or a gap.
- `Est.utrP*` is a penalty introduced to try to limit the extension of UTR beyond the frontier of transcribed evidence when there is some. For a defined length, the adequate UTR states that precede or follow an uninterrupted stretch of transcribed evidence will be penalized by the logarithm of this parameter.
- `Est.utrM` gives the number of UTR nucleotides that will be penalized using the previous penalty on the border of transcribed evidence.
- `Est.StrongDonor` gives a threshold T on donor strength inside intronless EST. If a given Donor with scores $a|b$ such that $\log(\frac{a}{b}) > T$ then the intronless EST is rejected because it goes through a very strong donor site. `Est.StrongDonor` value is included in]0;1[.
- `Est.MinDangling` gives the minimum length of the first and last match region in a genomic spliced alignment. If the length is below this, then it is assumed to be a false match and it is ignored.
- `Est.MaxIntron` gives the maximum length for the first and last gap (representing introns) in a spliced alignment. If the length exceeds this maximum, then the corresponding regions are ignored in the alignment (the long gap and the dangling hit).
- `Est.MaxInternalIntron` gives the maximum length for any gap (representing an intron) in a spliced alignment. If the length exceeds this maximum, then the corresponding gap is ignored as a probable bad spliced alignment or chimeric data.
- `Est.mRNAOnly` In the default mode (1), the plugin reads transcribed sequences which are mRNA fragments or complete cDNA, so the plugin always penalizes ncRNA states. Set this parameter to 0 to read non coding RNA fragments too: in this case, only ncRNA tracks which are incompatible with the alignment will be penalized.

The sensor is also capable of a postprocessing analyse described below and activated either by the `-E` argument or by setting the value 1 or 2 for the parameter `Est.PostProcess` in the parameter file.

The `Sensor.Est` is loaded last because it has the highest priority. This is important since the sensor actually uses the information provided by other sensors (splice site prediction sensors) that then have to be loaded before.

Here is an example of Est parameters definition.

```
Est.PostProcess[0]       0      # 0 1 OR 2
Est.PPNumber[0]          5      # For PostProcess = "2"
Est.estP*[0]             -0.4
Est.estM[0]              6
Est.utrP*[0]             0.35
Est.utrM[0]              5
Est.SpliceBoost*[0]      0.0
Est.StrongDonor[0]       0.95
Est.MinDangling[0]       10
Est.MaxIntron[0]         15000
Est.MaxInternalIntron[0] 15000
Est.mRNAOnly[0]          1
Est.FileExtension[0]     .est
Sensor.Est.use           1      # Use EST sensor
Sensor.Est               20     # Sensor priority: the highest one
```

Native input files format The plugin reads information from the file whose name is the concatenation of the sequence file name and the `Est.FileExtension` parameter. Each line of the input file is composed of 8 fields:

<b^S> <e^S> <s^S> <x> <b^S> `<est name>` <bq^S> <eq^S>

where:

- b^S and e^S are the begin and the end of the similarity S on the genomic sequence,
- s^S is the score of the similarity S (number of identical bases)
- x is unused for now
- b^S is the strand where the similarity occurs (forward = 0, reverse = 1). This information is not used anymore by the plugin which decides the strand of similarity by itself if there is anough information.
- `<est name>` is the name of the EST/cDNA sequence. Each sequence MUST have a unique name.
- <bq^S> <eq^S> are the begin and the end of the similarity S on the query (EST/cDNA) sequence.

The lines in the file must be ordered by sequence name first (all the hits of a given EST are put together) and by increasing <bq^S> <eq^S>.

Here is an example for the `SYNO_ARATH.tfa.est` file:

```
  32    421 1844 0 0 ATAJ644    1  390
 514    582 1844 0 0 ATAJ644  391  459
 699    809 1844 0 0 ATAJ644  460  570
 914   1018 1844 0 0 ATAJ644  571  675
1271   1408 1844 0 0 ATAJ644  676  813
1522   1602 1844 0 0 ATAJ644  814  894
1694   1771 1844 0 0 ATAJ644  895  972
1853   1921 1844 0 0 ATAJ644  973 1041
2014   2088 1844 0 0 ATAJ644 1042 1116
2181   2264 1844 0 0 ATAJ644 1117 1200
2360   2446 1844 0 0 ATAJ644 1201 1287
2712   2882 1844 0 0 ATAJ644 1288 1458
2966   3092 1844 0 0 ATAJ644 1459 1585
3189   3447 1844 0 0 ATAJ644 1586 1844
  32    375 347 0 0 AT00622    1  347
3071   3092 256 0 1 AI994358    1   22
3189   3421 256 0 1 AI994358   23  256
```

All the hits of the ATAJ644 are clustered together and sorted with increasing <bq^S> <eq^S> . In practice, this file can be directly constructed from an EST/cDNA bank and the sequence using a modified version of `sim4`. This version outputs splices alignements in the correct format (using the flag `A=6`) and only outputs hits with a coverage of more than 80% and with a similarity either than 90%.

A patch file `sim4.patch` is available in the plugin source directory as well as an awk script that put a FASTA sequence bank in a pure sim4 format (no upper case, no degenerated code). This seems useless on recent sim4 versions.

Gff3 input file format The gff3 input mode is activated by setting the value `GFF3` for the parameter `Est.format` in the parameter file. The plugin reads its information from the file whose name is the concatenation of the sequence file name, the `Est.FileExtension` parameter and the `.gff3` extension. Each line is as follows:

`<sequence name> <program> <sofa feature>` <b^S> <e^S> <v^S> `<strand> <.>` `ID=...;Target=<est name>` <bp^S> <ep^S> `<est strand>;score_hit=`<s^S>

Accepted features (third column):

- SO:0000668 or EST_match

Gff3 attributes specifications:
Required attributes:

- ID
- Target

Optional attributes:

- score_hit : normalized score.

Here an extract of : seq14ac002535g4g5.tfa.est.gff3

```
seq14     gth     EST_match     3261     4698     .     -     .     ID=EST_match:seq14.1;
seq14     gth     EST_match     3261     3332     0     -     .     ID=EST_match:seq14.1.1;Target=AV537418 1 72 +;score_hit=636;Parent=EST_match:
seq14     gth     EST_match     3411     3494     0     -     .     ID=EST_match:seq14.1.2;Target=AV537418 73 156 +;score_hit=636;Parent=EST_matc
seq14     gth     EST_match     3583     3657     0     -     .     ID=EST_match:seq14.1.3;Target=AV537418 157 231 +;score_hit=636;Parent=EST_mat
seq14     gth     EST_match     4209     4301     0     -     .     ID=EST_match:seq14.1.4;Target=AV537418 232 324 +;score_hit=636;Parent=EST_mat
seq14     gth     EST_match     4387     4698     0     -     .     ID=EST_match:seq14.1.5;Target=AV537418 325 636 +;score_hit=636;Parent=EST_mat
```

Filtering input information The EST information goes through a complex filtering process. First all hits are loaded. Successive hits of a same sequence are considered as a single alignment. For every spliced alignement, the plugin checks if a splice site of the correct type as been predicted near the border of each gap (less than `Est.estM` bases aways of the border). This is checked on each strand. If a strand does not contain the necessary splice sites, then it is considered as impossible. If neither strand contains adequate splice sites, the sequence is discarded (filtered).

All remaining alignments are sortered by 1) decreasing number of detected gaps then by 2) length (this tends to put cDNA or spliced EST alignments first) and 3) by the alphabetical order of the sequence names (to avoid sorting ambiguities). Any sequence that is inconsistent with previous sequence in this order (in the sense that they indicate that a given nucleotide is part of an intron, resp. exon, while a previous sequence indicates that the nucleotide is part of an exon (resp. intron) is discarded (filtered).

Any unspliced sequence that crosses a donor site that is predicted with a sufficiently strong confidence (See `Est.StrongDonor*`) is filtered out.

Integration of information Using filtered EST/cDNA sequence that are all consistent, every nucleotide can either be located:

Hit inside a matching segment that can occur on the forward strand on the reverse strand or both (as identified during filtering).

Gap or inside a gap segment that can occur on the forward strand on the reverse strand or both (as identified during filtering).

None or otherwise outside of any existing hit or gap (or less than `Est.estM` bases aways of the border of such a segment)

For a Hit: all intronic and intergenic tracks as well as UTR and exonic tracks on a strand incompatible with the hit are penalized with `Est.estP*`.

For a Gap: all exonic and intergenic tracks as well as UTR and intronic tracks on a strand incompatible with the hit are penalized with `Est.estP*`.

When no information exists (None), the UTR tracks are penalized by `Est.utrP*` if and only if there is a Hit evidence at less than `Est.utrM` bases away.

Post analysis The correspondence between transcribed sequence information and prediction is analyzed if the `-E` argument is activated or if the `Est.PostProcess` parameter value is `1` or `2`.

- `Est.PostProcess = 1`: EST/cDNA are compared to the prediction

  For each predicted transcript (from 5'UTR to 3'UTR), each available EST/cDNA sequence that overlaps the transcript is compared to the prediction. The transcribed sequence is then classified as:

  - Filtered (if it was filtered in the initial filtering process)
  - Inconsistent (if it is incompatible with the prediction)
  - Full transcript Support (if it is completely consistent with the predicted transcript on all the predicted transcript length)
  - Full Coding Support (if it is completely consistent with the predicted CDS on all the predicted CDS length, from start to stop codon)
  - Support (if it is otherwise consistent with the prediction)

  Finally, for each predicted transcript (from 5'UTR to 3'UTR) and predicted CDS (from start to stop), the number of predicted nucleotides that are supported by existing transcripts (filtered or not) is reported.

- `Est.PostProcess = 2`: the prediction is compared to the EST/cDNA

  For each predicted coding exon, the percentage of nucleotides supported by a transcribed sequence is displayed. This count includes all EST/cDNA, including those filtered. Overlapped transcribed sequences are displayed in the last column. The `Est.PPNumber` parameter is the maximum number of displayed transcribed sequences per exon.

Graph Non filtered transcribed evidence are plotted as blue horizontal blocks (hits) separated by thin blue lines (gap) on the intronic tracks. If the strand of the transcription has been identified during filtering, the blocks and lines occurr only on the corresponding IR or IF track.

Filtered sequences are plotted as gray blocks and lines just above (below) the forward (reverse) intronic tracks.

1.5.4.3 `Sensor.Homology`

Description This sensor is intended to take into account information from one or more homologous DNA sequences, usually genomic sequences from other species (inter-genomic homology), other sequences from the same genome (intra-genomic homology), or transcript sequences from normalized cDNA sets. The underlying general idea is that during evolution, functional genomic regions (*e.g.* exons) tend to be more conserved than non-functional ones (*e.g.* introns). The sensor increases the coding score of a genomic position that is included in a conserved region.

Homology detection has to be performed beforehand by the `TBlastX` software. Resulting alignment files require a specific format (see below).

The sensor is activated by either:

- the `-t` argument
- a value higher than 0 for the parameter `Sensor.Homology.use` in the parameter file.

Here is an example of Homology parameters definition:

```
Homology.TblastxP*[0]      0
Homology.TblastxB*[0]      0.0595
Homology.protmatname[0]    BLOSUM80
Homology.MaxHitLen[0]      15000
Homology.FileExtension[0] .tblastx
Sensor.Homology.use        1         # Use Homology sensor
Sensor.Homology            1         # Sensor priority
```

`Homology.protmatname` is the name of the file containing the amino acid substitution score matrix used to measure the base homology score at the proteic level, before scaling (standard text format). `Homology.TblastxP` and `Homology.TblastxB` parameters are used to scale the information given by homology regions. For more details, please refer to the publication "EuGÈNE'HOM: a generic similarity-based gene finder using multiple homologous sequences" (Foissac *et. al*, *Nucleic Acids Res.*, 2003, 31(13):3742-5).

Native input files format The plugin reads information from the file whose name is the concatenation of the sequence file name and the `Homology.FileExtension` parameter. The file describes a collection of similarities sorted by subject sequence and by position on the query sequence.

These files are obtained by blasting with `TBlastx` the genomic sequence (the query) against a set of other DNA sequences, and by parsing the results with the `ParseBlastXML.pl` script.

The TBlastX is launched with the command:

```
blastall -p tblastx -i QUERY_GENOMIC_SEQUENCE_FASTA -d
DATABASE_MULTIFASTA_FILE -F T -M SUBSTITUTION_MATRIX_FILE -e 1e-6 -b
50000 -m 7 > TEMPORY_BLAST_RESULT_FILE
```

Note: in order to reduce the number of "phantom frame" hits, the amino acid substitution matrix (e.g. BLOSUM62) should be modified by setting every score involving a STOP codon (lines and column noted with a star *) to a huge penalty (e.g. -500).

and the final `.tblastx`$< i >$ files are obtained with:

```
ParseBlastXML.pl TEMPORY_BLAST_RESULT_FILE > QUERY_GENOMIC_SEQUENCE_FASTA.tblastx
```

For more explanation, see the README file in the directory `eugene/src/SensorPlugins/Homology/GetData`.

One similarity S is described per line.

The format of a line is similar to the ".blast" file format (BlastX sensor) with an additional column displaying the translated sequence (amino acid alphabet) of the subject matching region:

<b^S> <e^S> <s^S> <v^S> <p^S> `<subject seq. name>` <bp^S> <ep^S> `<AA_SEQ>`

where:

- b^S and e^S are the begin and the end of the similarity S on the query sequence,
- s^S is the score of the similarity S,
- v^S is the e-value given by TBlastX and ignored by EUGÈNE,
- p^S is the phase: +1, +2, +3, -1, -2, -3,
- bp^S and $ep^S$are the begin and the end of the similarity S on the subject sequence,
- AA_SEQ is the amino acid sequence translated from the subject nucleic region.

Here is an example of the format:

```
831 878 51 7e-26 -3 ATHA10A_809_856 809 856 TLQLHGRRYVETTVFV
828 878 48 1e-20 +3 ATHA10A_806_856 806 856 RDKHRCFHVSSAMKLEG
1572 1652 109 7e-114 -3 ATHA10A_1349_1429 1349 1429 IPWSNLLELKSTPMILEAPAILAPSAA
1738 1821 108 1e-153 +1 ATHA10A_1493_1576 1493 1576 FDMLLAAKEFGVTECVNPKDHDKPIQQV
830 877 86 1e-153 +2 ATHA10A_808_855 808 855 GQTPLFPRIFGHEAGG
590 625 44 1e-20 +2 ATHA10A_562_597 562 597 LQLLWHGKPESH
```

Gff3 input file format The gff3 input mode is activated by setting the value `GFF3` for the parameter `Homology.format` in the parameter file. The plugin reads its information from the file whose name is the concatenation of the sequence file name, the `Homology.FileExtension` parameter and the `.gff3` extension. Each line reads as follows:

`<sequence name> <program> <sofa feature>` <b^S> <e^S> <v^S> `<strand> <.> ID=...;Target=`
`name>` <bp^S> <ep^S> `<target strand>;frame_hit=`<p^S>`;score_hit=`<s^S>

Gff3 attributes specifications:
Required attributes:

- ID
- Target
- target_sequence : amino acid sequence.

Optional attributes:

- frame_hit represents the blast frame. This information can be omitted, but if it's specified, eugene will check it is consistent with the similarity positions and reject the information otherwise.
- score_hit : normalized score.

Here an extract of `seq14ac002535g4g5.tfa.tblastx.gff3`:

```
seq14       tblastx      match       129       236       2e-25       +       .       ID=tblastx:seq14.1;Dbxref=tblastx:ATKIN2_100_207;Target=ATKIN2_100_207 100
seq14       tblastx      match       86       130       3e-63       -       .       ID=tblastx:seq14.2;Dbxref=tblastx:ATKIN2_102_58;Target=ATKIN2_102_58 102 58
```

Filtering input information Note that similarities which have a length higher than `Homology.MaxHitLen` parameter value are rejected. A message "Similarity of extreme length rejected. Check tblastx file <NAME>" is printed to alert the user.

Integration of information The TBlastX search returns a set of High Scoring Pairs (HSP), each in a given frame. All pairs of HSP which overlap and are in the same frame are clustered together in so-called "HSP contigs". To associate an homology score to a given nucleotide n_i in the context of a coding region, we consider (if it exists), the single HSP contig HC that overlaps the nucleotide in the sequence and which is in the same frame. Let n be the maximum number of HSPs in the cluster that overlap a single position. Let $c(n_i)$ be the codon that contains n_i in the sequence, for each HSP h in the cluster that overlaps the codon $c(n_i)$, we define $S(c(n_i), h)$ to be the matrix substitution score for the amino acid coded by $c(n_i)$ in the HSP alignment. This score is considered as equal to zero for non overlapping HSP. The homology score for the nucleotide in the context of the coding region considered is defined as:

$$HS(n_i) = \frac{1}{n} \sum_{h \in HC} S(c(n_i), h)$$

The resulting score provided by the sensor after scaling is equal to

$$\texttt{Homology.TblastxB}\ .HS(n_i) + \texttt{Homology.TblastxP}$$

Post analyse None.

Graph HSP clusters are represented as grey blocks whose thickness is proportional to the number of hits at a given position and whose darkness is proportional to the homology score at this position.

1.5.4.4 `Sensor.MarkovConst`

Description A simulated content sensor that gives constant probabilities (as indicated in the `MarkovConst-.Coding*`, `MarkovConst.Intron*`, `MarkovConst.IntronUTR*`, `MarkovConst.UTR5*`, `Mark-ovConst.UTR3*`, `MarkovConst.UIR*` and `MarkovConst.Inter*` parameters). to all positions for each region type. As the MarkovIMM sensor, the plugin is controlled by two further parameters: `MarkovConst.minGC` and `MarkovConst.maxGC` which indicate the GC scope of the contents sensor. If the GC% of the sequence is out of the scope, the plugin will give an equal null loglikelihood to all types of regions.

Used for testing purposes and for simulating the exponential length distributions of HMM.

Here is an example of MarkovConst parameters definition.

```
MarkovConst.Coding*         1.0
MarkovConst.Intron*         1.0
MarkovConst.IntronUTR*      0.98
MarkovConst.UTR5*           0.99
MarkovConst.UTR3*           0.99
MarkovConst.UIR*            0.99
MarkovConst.RNA*            0.99
MarkovConst.Inter*          1.0
MarkovConst.affectedStrand 0
MarkovConst.minGC[0]        0
MarkovConst.maxGC[0]        100
Sensor.MarkovConst.use      1   # Use MarkovConst sensor
Sensor.MarkovConst          1      # Sensor priority
```

Input files format No input files needed.

Integration of information If the GC% of the sequence handled is between `MarkovConst.minGC` and `MarkovConst.maxGC` then in every position, in all possible states, the prediction is penalized by the logarithm of the corresponding parameter: `MarkovConst.Coding*`, `MarkovConst.Intron*`, `MarkovConst.IntronUTR*`, `MarkovConst.UTR5*`, `MarkovConst.UTR3*`, `MarkovConst.UIR*`, `MarkovConst.RNA*` and `MarkovConst.Inter*`.

The `MarkovConst.affectedStrand` parameter allows to specify the affected strand and thus to turn off the prediction on the opposite strand. To predict only on forward strand set `MarkovConst.affectedStrand` parameter value to 1; to predict only one reverse one set it to -1, and to predict on both strands let the value to 0.

Post analyse No post analyse.

Graph No plotting.

1.5.4.5 `Sensor.MarkovIMM`

Description This plugin injects coding/intronic/utr/rna/intergenic likelihood as modeled by interpolated Markov models (introduced in Glimmer, see S. Salzberg, A. Delcher, S. Kasif, and O. White.*Microbial gene identification using interpolated Markov models* Nucleic Acids Research 26:2 (1998), 544-548). These models are defined in a so-called matrices file (located in the EUGENEDIR/models directory) whose name is indicated by the `MarkovIMM.matname` parameter. Depending on the matrices file, this may contain IMM for exons, introns and intergenic data and also optionnally 5' and 3' UTR regions. If these 2 last IMMs are absent from the matrices file, intronic models are used for UTR in eukaryote mode, and intergenic model is used for UTR in prokaryote mode.

The plugin is controlled by two further parameters: `MarkovIMM.minGC` and `MarkovIMM.maxGC` which indicate the GC scope of the matrices. If the GC% of the sequence is out of the scope, the plugin will give an equal null loglikelihood to all types of regions.

By instanciating multiple `MarkovIMM` plugins (see section 1.5), this enables the use of several IMM according to the GC% of the input sequence.

The intergenic track can receive score in three possible ways according to the `MarkovIMM.IntergenicModel` parameter value :

0 In this case, a O^{th} order Markov model that is directly estimated from the frequencies in the sequence is used.

1 The intergenic IMM matrix is directly used on the forward strand. This model will give a slightly different score to the sequence and to its reverse complement but is simple and works well on some organisms.

2 The intergenic model is used on both strands and the mean of the two prababilities (forward and reverse) is used as the estimation. This is the default model to use. It has the advantage of giving the same score to a sequence and its reverse complement.

The npcRNA track can receive score in two possible ways according to the `MarkovIMM.npcRNAModel` parameter value :

1. `gc` In this case, a O^{th} order Markov model that is directly estimated from the frequencies in the sequence is used.

2. `numeric value` In this case, a constant score, which is the log of this value.

The sensor is activated by either:

- the `-m` argument followed by the filename of the set of Markov models.
- the value 1 for the parameter `Sensor.MarkovIMM.use` in the parameter file.

Here is an example of MarkovIMM parameters definition.

```
MarkovIMM.matname     Ara2UTR.mat
MarkovIMM.minGC       0
MarkovIMM.maxGC       100
MarkovIMM.IntergenicModel  2
MarkovIMM.npcRNAModel  gc
MarkovIMM.maxOrder  8
Sensor.MarkovIMM.use 1
Sensor.MarkovIMM      1      # Sensor priority
```

Input files format No input files needed beyond the IMM matrix file.

Integration of information On the forward intronic, exonic and UTR tracks, the probability that the nucleotide at position i appears given the nucleotides that follow him is fetched in the IMM matrix file. The logarithm of this probability is used as a penalty on the corresponding track.

For reverse tracks, the same process is used but the probability used is the probability that the nucleotide at position i appears given the nucleotides that precede him.

For the intergenic track, the probability used is the mean of the probability that the nucleotide at position i appears given the nucleotides that precede him and the probability that the nucleotide at position i appears given the nucleotides that follow him. This guarantees that a sequence and its reverse complement will receive the same weights exactly.

Post analyse No post analyse.

Graph The likelihood of a subsequence of width `Output.window` is computed for each IMM model and normalized over all these. The corresponding normalized likelihood is plotted as a thin black line on each track of the graphical output.

1.5.4.6 `Sensor.MarkovProt`

Description This plugin injects coding/non coding likelihood as modeled by proteic Markov models. These models are defined in a matrices file (located in the directory specified by the `EuGene.PluginsDir` parameter) whose name is indicated by the `MarkovProt.matname` parameter. The order of the Markov model must be given in `MarkovProt.maxorder` while the actual order to use is set by `MarkovProt.order`.

The plugin is controlled by two further parameters: `MarkovProt.minGC` and `MarkovProt.maxGC` which indicate the GC scope of the matrices. If the GC% of the sequence is out of the scope, the plugin will give an equal null loglikelihood to all types of regions.

The sensor is activated by either:

- the `-M` argument followed by the filename of the set of models.

- the value 1 for the parameter `[Sensor.MarkovProt.use` in the parameter file.

Here is an example of MarkovProt parameters definition.

```
MarkovProt.matname      SwP41.noFragm.mininfo1.order2.bin
MarkovProt.minGC        0
MarkovProt.maxGC        100
MarkovProt.maxorder     2
MarkovProt.order        2
Sensor.MarkovProt.use   1    # Use MarkovProt sensor
Sensor.MarkovProt       1       # Sensor priority
```

Input files format No input files needed beyond the markov matrix files.

Integration of information For coding tracks, assuming a uniform codon usage, the probability of the coding tracks is decomposed as the product of choosing a codon and then emitting the corresponding amino acid in the corresponding phase. The logarithm of the probability is used for weighting.

For other tracks, a simple GC% model is used to compute a background probability. The logarithm of the probability is used for weighting.

Post analyse No post analyse.

Graph Same as in the MarkovIMM plugin.

1.5.4.7 `Sensor.Repeat`

Description The plugin allows to exploit the output of repeated sequences detector such as RepeatMasker by penalizing exonic, inronic or UTR states when repeats are detected.

The sensor is activated by either :

- the `-r` argument
- the value 1 for the parameter `Sensor.Repeat.use` in the parameter file.

The penalties used when a repeat exists are `Repeat.IntronPenalty*`, `Repeat.ExonPenalty*` and `Repeat.UTRPenalty*` respectively.

Here is an example of Repeat parameters definition.

```
Repeat.UTRPenalty*      0.0
Repeat.IntronPenalty*   0.1
Repeat.ExonPenalty*     1.0
Sensor.Repeat.use       1    # Use Repeat sensor
Sensor.Repeat           1       # Sensor priority
```

Native input files format The file with a `.ig` suffix is needed. Each line of the file contains the beginning and the end of a region detected as a repeat. The positions must be sorted in increasing positions. Such a file can be obtained by eg. reformatting RepeatMasker output.

Here is an extract from a typical `.ig` file:

```
4800    5006
22494   22758
22703   22772
22841   23017
22929   23017
29433   29703
[...]
```

Gff3 input file format The gff3 input mode is activated by setting the value `GFF3` for the parameter `Repeat.format` in the parameter file. The plugin reads the file which name is derived from the sequence name by adding the `.ig.gff3` extension.

Accepted features (third column):

- SO:0000657 or repeat_region

If the feature used isn't one of those, the line will be rejected. Here an extract of `seq14ac002535g4g5.tfa.ig.gff3`.

```
seq14       RepeatMasker        repeat_region       1       100        .        .        .        ID=repeat_region:seq14.1;
```

Filtering input information No filtering.

Integration of information For exonic, intronic and UTR tracks, all positions that occur in a repeat interval as reported in the `.ig` file are penalized using the corresponding `Repeat.IntronPenalty*`, `Repeat.ExonPenalty*` and `Repeat.UTRPenalty*` penalties.

Post analyse No post analyse.

Graph Repeat intervals are visualized as grey blocks in the intergenic track.

1.5.4.8 `Sensor.NStretch`

Description A content sensor that penalizes theprediction of anything but intergenic regions for stretchs of 'N' longer than `NStretch.maxLengthWithoutPenalty`.

Here is an example of NStretch parameters definition.

```
NStretch.stretchPenalty 1.0
NStretch.maxLengthWithoutPenalty      5000
Sensor.NStretch.use  1   # Use Stretch sensor
Sensor.NStretch      1   # Sensor priority
```

Input files format No input files needed.

Integration of information All non-intergenic predictions are penaluzed at positions that occur in a stretch of N are penalized using the `NStretch.stretchPenalty` penalty.

Post analyse No post analyse.

Graph No plotting.

1.5.5 `Mixed signal/content plugins`

1.5.5.1 `Sensor.AnnotaStruct`

Description The sensor allows to seamlessly modify EUGÈNE underlying graph weighting using a small langage that can directly modify the weights of signals and contents edges in the graph. The plugin offers both high-level entries and low-level entries in either a sloppy GFF-like format or a strict GFF3-compliant format.

The high-level entries allow to take into account information on:

- **transcribed sequences** (involving exons, introns, UTR, transcription start and transcription stop and splice sites) that may come from alignment of transcribed sequences (using spliced alignment algorithms such as sim4 or PASA).
- **CDS** (involving exons, introns, translation start, translation stop and splice sites) that may come from other gene predictors that may predict CDS (either *ab initio* or homology based predictors).
- **ncRNA** (involving non protein coding RNA region, ncRNA transcription start and ncRNA transcription stop).

The high-level entries are actually automatically expanded in elementary (low-level) information as the plugin reads the data. The way the expansion takes place is user-controllable through parameters.

Compared to the `Est` plugin, there is no data filtering performed here which means that the plugin should rather be used on consistent and fairly reliable data (eg. on existing gene predictions, cDNA or EST cluster alignements rather than simple EST alignments that would be better handled using the `Est` plugin).

The low-level entries allow to directly modify every edge of the underlying prediction graph of EUGÈNE as the (now obsolete) `User` plugin allowed. The weights of all signals edges (transcription start and stop, translation start and stop, splice sites, insertions and deletions, ncRNA transcription start and stop) and contents edges (exons, introns, UTR, UTR introns, intergenic regions and ncRNA) can be directly modified using this plugin.

To activate the sensor, put the number of AnnotaStruct instances you want to create to the parameter `Sensor.AnnotaStruct.use` in the parameter file.

Here is an example of AnnotaStruct parameters definition :

```
AnnotaStruct.FileExtension[0]     gff
AnnotaStruct.TranscriptFeature[0]  mRNA
AnnotaStruct.Exon*[0]             1
AnnotaStruct.Intron*[0]           2
AnnotaStruct.CDS*[0]              3
AnnotaStruct.npcRNA*[0]           2
AnnotaStruct.Intergenic*[0]       0
AnnotaStruct.StartType[0]         p
AnnotaStruct.Start*[0]            0.1
AnnotaStruct.StopType[0]          p
AnnotaStruct.Stop*[0]             0.1
AnnotaStruct.AccType[0]           p
AnnotaStruct.Acc*[0]              0.1
AnnotaStruct.DonType[0]           p
AnnotaStruct.Don*[0]              0.1
AnnotaStruct.TrStartType[0]       p
AnnotaStruct.TrStart*[0]          0.1
AnnotaStruct.TrStopType[0]        p
AnnotaStruct.TrStop*[0]           0.1
AnnotaStruct.TrStartNpcType[0]    p
AnnotaStruct.TrStartNpc*[0]       0.1
AnnotaStruct.TrStopNpcType[0]     p
AnnotaStruct.TrStopNpc*[0]        0.1

Sensor.AnnotaStruct.use       1         # Use one AnnotaStruct sensor instance
Sensor.AnnotaStruct          1         # Sensor priority
```

Native GGF-like input files format The plugin reads a GFF format file. Each line in this file forms an elementary information which is directly interpreted by the plugin independently of other lines. A GFF line is formed by a sequence of separated fields: sequence name, source, feature, start, end, score, strand and frame. The sequence name and source fields are ignored by the plugin and can be set to user informative values.

Each line may either represent a high-level or a low-level information. Low-level informations use specific features for specifying which signals and contents edges should be modified. For signals, the following features are recognized:

- `trStart`: for transcription starts.
- `trStop`: for transcription stops.
- `start`: for translation starts (ATG).
- `stop`: for translation stops.
- `acc`: for acceptor splice sites.
- `don`: for donor splice sites.
- `ins`: for insertion (frameshift).
- `del`: for deletion (frameshift).
- `trStartNpc`: for ncRNA transcription starts.
- `trStopNpc`: for ncRNA transcription stops.

In this case, the start and the strand field are used to indicate the signal position. The score field is used to indicate the weight that will be used to modify the existing weight. It is either a floating point value between `-1e999` and `1e999` (that match $-\infty$ and ∞ respectively in the format used) or a floating point between 0.0 and 1.0 preceded by the letter p (like probability).

1. In the first case, the score indicated is directly added to the weight of the signal edge (that corresponds to the fact that the signal is used). The other signal edge is unmodified.
2. In the second case, the score s that appears after the p is treated as a (conditional) probability. The edge that corresponds to the fact that the signal is used receive a weight $\log(s)$ and the other edge $\log(1-p)$.

For contents edges, the following features are recognized:

- `interg`: for non transcribed regions

- `exon`: for coding exons.
- `intron`: for introns separating coding exons.
- `utr5`: for 5' UTR (untranslated terminal regions).
- `utr3`: for 3' UTR.
- `utr`: for both 5' or 3' UTR.
- `intronutr`: for UTR introns.
- `ncrna`: for non protein coding RNA.

The start and end fields together with the strand field delimit the region considered. All corresponding contents edges will be modified by the weight indicated in the score field.

High-level information may either be used to express knowledge about potential *transcribed sequences* or potential *coding sequences*. For information about CDS regions, the following features may be used:

- `E.Init`: for an initial exon, this will automatically expand in the weight modification of a translation start and a donor site at the corresponding extremities on the indicated strand (using parameters `AnnotaStruct.Start*` and `AnnotaStruct.Don*` respectively as weights) and the contents modification for the exon in the corresponding frame and strand (using the score indicated in the `AnnotaStruct.CDS*` parameter).
- `E.Intr`: for an intermediary exon, this will automatically expand in the weight modification of a donor and an acceptor site at the corresponding extremities on the indicated strand (using parameters `AnnotaStruct.Don*` and `AnnotaStruct.Acc*` respectively as weights) and the contents modification for the exon in the corresponding frame and strand (using the score indicated in the `AnnotaStruct.CDS*` parameter).
- `E.Term`: for a terminal exon, this will automatically expand in the weight modification of an acceptor and a stop signal at the corresponding extremities on the indicated strand (using parameters `AnnotaStruct.Acc*` and `AnnotaStruct.Stop*` respectively as weights) and the contents modification for the exon in the corresponding frame and strand (using the score indicated in the `AnnotaStruct.CDS*` parameter).
- `E.Sngl`: for a single exon gene, this will automatically expand in the weight modification of a translation start and stop signal at the corresponding extremities on the indicated strand (using parameters `AnnotaStruct.Start*` and `AnnotaStruct.Stop*` respectively as weights) and the contents modification for the exon in the corresponding frame and strand (using the score indicated in the `AnnotaStruct.CDS*` parameter).
- `UTR5`, `UTR3`, `UTR`: although not part of the CDS, some gene predictors may predict UTR (non coding part of exons). These 3 features allow to inject this information by respectively reweighting a transcription start, stop or both using the corresponding `AnnotaStruct.TrStart*`, `AnnotaStruct.TrStop*` parameters and then by reweighting the UTR5, UTR3 or both contents edges (using the `AnnotaStruct.CDS*` parameter).
- `Intron`: equivalent to `intron` except that the weight used comes from the `AnnotaStruct.CDS*` parameter).

For information about transcribed sequences, the following features are recognized:

- `E.Any`: any exon in the biological sense *i.e.* either an exon or a UTR in the EuGène sense. Frame is typically unknown (in this case, all coding frame in the indicated strand are considered). The corresponding contents region are modified accordingly to the `AnnotaStruct.Exon*` parameter.

- `E.First`: the first biological exon (containing UTR and possibly part of CDS too). A transcription start signal is weighted according to the `AnnotaStruct.TrStart*` parameter value. The UTR5 and coding exon contents edges are reweighted according to the `AnnotaStruct.Exon*` parameter value.
- `E.Last`: the last biological exon. A transcription stop signal is weighted according to the `AnnotaStruct.TrStop*` parameters value. The UTR3 and coding exon contents edge are reweighted according to the `AnnotaStruct.Exon*` parameter value.
- `E.Extreme`: used for a biological exon on the extremity (either first or last). A transcription start and stop are generated at each respective extremities according to the `AnnotaStruct.trStart*` and `AnnotaStruct.trStop*` parameter values. The UTR5, UTR3 and coding exon contents edges are reweighted according to the `AnnotaStruct.Exon*` parameter value.
- `ncRNA`: a non protein coding RNA (at the moment, EuGene doesn't predicted ncRNA intron). A non protein coding transcription start and stop are generated at each respective extremities according to the `AnnotaStruct.trStartNpc*` and `AnnotaStruct.trStopNpc*` parameter values. The ncRNA content edge is reweighted according to the `AnnotaStruct.npcRNA*` parameter value.
- `Intron.Any`: ?

Here is a high-level CDS based example:

```
ATSYNO FGENESH E.Init  3 33 0 + 3
ATSYNO FGENESH E.Term 45 75 0 + 3
```

Gff3 input files format The gff3 input mode is activated by setting the value `GFF3` for the parameter `AnnotaStruct.format` in the parameter file. The plugin reads its informations from a file named with the sequence name and an extension `AnnotaStruct.FileExtension` followed by `.gff3` In gff3 mode, you can't describe feature level as in natif mode.

If you want to read scores or probabilities from file you have to set parameters score to "i" (for "inline") in the parameter file. Content data will always be interpreted as score.

```
AnnotaStruct.Exon*             i  # interpreted as score
AnnotaStruct.Intron*           i  # interpreted as score
AnnotaStruct.CDS*              i  # interpreted as score
AnnotaStruct.npcRNA*           i  # interpreted as score
AnnotaStruct.Intergenic*       i       # interpreted as score
AnnotaStruct.StartType         p  # p: probability  s: score
AnnotaStruct.Start*            i
AnnotaStruct.StopType          p  # p: probability  s: score
AnnotaStruct.Stop*             i
AnnotaStruct.AccType           p  # p: probability  s: score
AnnotaStruct.Acc*              i
AnnotaStruct.DonType           p  # p: probability  s: score
AnnotaStruct.Don*              i
AnnotaStruct.TrStartType       p  # p: probability  s: score
AnnotaStruct.TrStart*          i
AnnotaStruct.TrStopType        p  # p: probability  s: score
AnnotaStruct.TrStop*           i
AnnotaStruct.TrStartNpcType    p # p: probability  s: score
AnnotaStruct.TrStartNpc*       i
AnnotaStruct.TrStopNpcType     p # p: probability  s: score
AnnotaStruct.TrStopNpc*        i
```

Here is the correspondance beetween GFF native AnnotaStruct features and GFF3 features. A direct translation between the two formats is not always possible (for example, low-level content information cannot be given directly in GFF3, but can be indirectly achieved by setting signal at zero, and using the transcribed sequence feature).

Each GFF feature is translated in a GFF3 feature (column 3 in the GFF3 file). Some may require an additional ontology term (specified in the column 9 with the key Ontology_term). The expected value in the column 3 and if an Ontology_term is required, the expected value in the column 9, are given below.

Low-level information:

- `trStart`: SO:0000315 (or TSS)
- `trStop`: SO:0000616 (or transcription_end_site)
- `start`: SO:0000318 (or start_codon).
- `stop`: SO:0000319 (or stop_codon)
- `acc`: SO:0000164 (or three_prime_cis_splice_site)
- `don`: SO:0000163 (or five_prime_cis_splice_site)
- `ins`: SO:0000366 (or insertion_site)
- `del`: SO:0000687 (or deletion_junction)
- `trStartNpc` and `trStopNpc` : no corresponding feature

High-level information:

Translated regions information

- `E.Init`: CDS (or SO:0000316) + Ontology_term=SO:0000196 (five_prime_coding_exon_region)
- `E.Intr`: CDS (or SO:0000316) + Ontology_term=SO:0000004 (interior_coding_exon)
- `E.Term`: CDS (or SO:0000316) + Ontology_term=SO:0000197 (three_prime_coding_exon_region)
- `E.Sngl`: CDS (or SO:0000316) + Ontology_term=SO:0005845 (single_exon)
- `UTR5`: five_prime_UTR (or SO:0000204)
- `UTR3`: three_prime_UTR (or SO:0000205)
- `UTR`: UTR (or SO:0000203)
- `Intron`: intron (or SO:0000188) + Ontology_term=SO:0000191 (interior_intron)

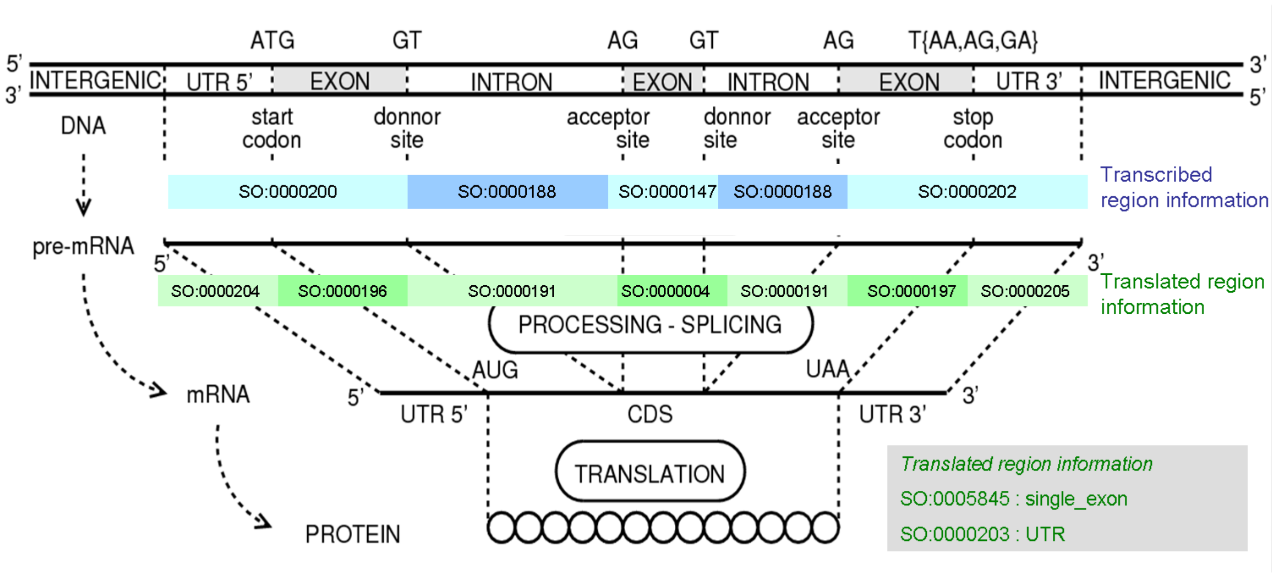
Transcribed region information

- `E.Any`: exon (or SO:0000147)
- `E.First`: exon (or SO:0000147) + Ontology_term=SO:0000200 (five_prime_coding_exon)
- `E.Last`: exon (or SO:0000147) + Ontology_term=SO:0000202 (three_prime_coding_exon)
- `E.Extreme`: exon (or SO:0000147) + Ontology_term=SO:0000200,SO:0000202
- `Intron.Any`: intron (or SO:0000188)
- `transcript`: transcript_region (or SO:0000833)

- `ncRNA`: ncRNA (or SO:0000655 or other SO terms derived from ncRNA, for instance rRNA (SO:0000252) or tRNA (SO:0000253). See `http://www.sequenceontology.org/wiki/index.php/Category:SO:0000655_!_ncRNA` for details.

Intergenic information

- `interg`: intergenic_region (or SO:0000605)



Here an extract of : seq14ac002535g4g5.tfa.gff.gff3

```
seq25        EuGene        five_prime_UTR        1        2787        0        +        .        ID=five_prime_UTR:seq25.0;
seq25        EuGene        CDS          2788         2836        0        +        0        ID=CDS:seq25.1;Ontology_term=SO:0000196
seq25        EuGene        CDS          8356         8471        0        +        2        ID=CDS:seq25.2;Ontology_term=SO:0000004
seq25        EuGene        CDS          8576         8667        0        +        1        ID=CDS:seq25.3;Ontology_term=SO:0000004
seq25        EuGene        CDS          9006         9061        0        +        0        ID=CDS:seq25.4;Ontology_term=SO:0000004
seq25        EuGene        CDS          9567         9655        0        +        1        ID=CDS:seq25.5;Ontology_term=SO:0000004
seq25        EuGene        CDS          10520         10535        0        +        1        ID=CDS:seq25.6;Ontology_term=SO:0000004
seq25        EuGene        CDS          10896         11134        0        +        1        ID=CDS:seq25.7;Ontology_term=SO:0000004
seq25        EuGene        CDS          11544         12005        0        +        2        ID=CDS:seq25.8;Ontology_term=SO:0000004
seq25        EuGene        CDS          12088         12900        0        +        0        ID=CDS:seq25.9;Ontology_term=SO:0000197
seq25        EuGene        three_prime_UTR        12901        14900        0        +        .        ID=three_prime_UTR:seq25.10;
```

For complete gene, ontology terms for CDS can be added on the fly by the plugin if the GFF3 includes Parent information to a transcript level feature. This feature is typically called "mRNA" or "transcript" in gene finders GFF3 output. If the `AnnotaStruct.TranscriptFeature` parameter is set accordingly, then the plugin will identify first, internal, terminal and single exon assuming the CDS features are in increasing position order.

Filtering input information No filtering beside syntax checking.

Integration of information The underlying graph edges are directly modified as indicated.

Post analyse No post analyse.

Graph No plotting.

1.5.5.2 `Sensor.IfElse`

Description This plugin is used to combine the predictions of two existing plugins. It listens to a first plugin. For each possible predictable item, if this plugin predicts something then this prediction is used. If the plugin does not predict anything, then the output of the second plugin is used.

The plugin needs only two parameters to be informed: `IfElse.SensorIf` and `IfElse.SensorElse` which indicate the names of the two slave plugins. The two slave plugins will be loaded with an instance number equal to one plus the instance number of the IfElse sensor itself (allowing for nested IfElse).

Here is an example of IfElse parameters definition which uses the NG2 Sensor if it predicts something or else the SPred sensor.

```
IfElse.SensorIf         NG2
IfElse.SensorElse       SPred
Sensor.IfElse.use       1    # Use IfElse sensor
Sensor.IfElse           1       # Sensor priority
```

In this case, since the IfElse is loaded as a first plugin (instance 0), the two slave plugins will be instanciated as instance number one. The parameters for the 2 plugins must therefore be suffixed by `[1]`.

Input files format No input files needed beyond those used by the slave sensors.

Filtering input information No filtering.

Integration of information The "If" plugin is called. For each of the possible information type (signal and contents), if nothing is predicted by it, the prediction of the second plugin is used instead.

Post analyse No post analyse beyond the post analyze in the slave plugins.

Graph Nothing beyond the plotting in the slave plugins.

1.5.5.3 `Sensor.Riken`

Description The plugin allows to exploit 5'/3' EST extracted from the extremities of full-length cDNA. This type of data was produced by the Riken institute for *Arabidopsis thaliana*. By mapping such EST to the genomic sequence, it is possible to know the positions where a gene (transcript) must start (5' side) and stop (3' side). The plugin assumes that this mapping has been done and that the coordinates of the extremities of the 5' and 3' EST of full-length clones have been determined before hand.

The sensor is activated by either :

- the `-R` argument
- the value 1 for the parameter `Sensor.Riken.use` in the parameter file.

The plugin is controled by several parameters, most of which control sanity checks (see below). The `Riken.RAFLPenalty*` parameter controls the amount of penalty used to force EuGÈNE to predict a gene on a region defined by a valid EST pair.

Here is an extract of Riken parameters definition :

```
Riken.Min_est_diff              100
Riken.Max_overlap               60
Riken.Max_riken_length          60000
Riken.Max_riken_est_length      3000
Riken.Min_riken_length          120
Riken.Min_riken_est_length      10
Riken.StrandRespect             0
Riken.RAFLPenalty*              -120
Sensor.Riken.use                1     # Use Riken sensor
Sensor.Riken                    7        # Sensor priority
```

Native input files format A file with extension `.riken` is read. Each line must contain the positions of the extremities of the match of the 5' EST then the name of the 5' EST, the same thing for the 3'EST and finally the name of the clone.

Here is an exert of a typical `.riken` file:

```
417757  418379  AV826766        418902  419330  AV796216        0907A18
341382  342036  AU235278        340748  341549  AU225941        1201K23
40318   40969   AV821185        38800   39323   AV781490        0208M10
309757  310341  AV830906        308043  308392  AV813791        0980B11
387624  388227  AU236666        387383  387834  AU227623        1514C21
148345  148909  AV822910        147090  147960  AV783778        0513A17
```

Gff3 input file format The gff3 input mode is activated by setting the value `GFF3` for the parameter `Riken.format` in the parameter file. The plugin reads the file which name is derived from the sequence name by adding the `.riken.gff3` extension. The parent link is very important, one parent must have two children (5' and 3'). The parent feature must be defined before the children.

Gff3 attributes specifications:
Required attributes:

- ID
- Target
- Parent for children feature
- Ontology_term : define 5' or 3' region for match_part feature (children).

Optional attributes:
is_full_length specify the type of data (eg : alignment with full length proteins)

- -1 not defined
- 0 not full length match EST or fragment proteins
- 1 like riken type : we know the 5' and 3'
- 2 match against full length proteins or cDNA

Here an extract of : seq14ac002535g4g5.tfa.riken.gff3

```
seq14       Riken       cDNA_match       1400       4718       .       .       .       ID=seq14.1
seq14       Riken       match_part       1910       4718       .       +       .       ID=seq14.1.1;Parent=seq14.1;is_full_length=1;Ontology_term=SO:0000200
seq14       Riken       match_part       1400       1500       .       +       .       ID=seq14.1.2;Parent=seq14.1;is_full_length=1;Ontology_term=SO:0000202
```

Filtering input information The plugin uses several parameters that control sanity checks on the input data.

- the `Riken.Max_riken_length` parameter controls the maximum length for a transcript. If an EST pair defines a transcript with a length of more than this number of base pairs, then it is ignored. A typical value is 60kb (for *Arabidopsis thaliana*).
- the `Riken.Min_riken_length` parameter controls the minimum length for a transcript. If an EST pair defines a transcript with a length lower than this number of base pairs, then it is ignored. A typical value is 120b (for *Arabidopsis thaliana*).
- the `Riken.Max_riken_est_length` parameter controls the maximum length of the genomic sequence matching one EST. If either the 5' or the 3' EST exceed this length, then the EST pair is rejected. A typical value is 3kb (for *Arabidopsis thaliana*).
- the `Riken.Min_riken_est_length` parameter controls the minimum length of the genomic sequence matching one EST. If either the 5' or the 3' EST are below this length, then the EST pair is rejected. A typical value is 10 bp (for *Arabidopsis thaliana*).
- the `Riken.StrandRespect` parameter controls whether the 5'/3' information available for the EST is taken into account or ignored. If this parameter is set to `0`, then the information is ignored and the prediction of a gene is "forced" in the region but with no constraint on the strand. Otherwise, and if the 5'/3' EST pair is separated enough to decide the strand, then the prediction of a gene is forced on the strand detected.
- the `Riken.Min_est_diff` parameter controls the minimum distance of separation between the 5' and 3' EST (computed as the sum of the distances of the left and right extremities of the two genomic sequences mapping the 2 EST) that is sufficient to deduce the strand of the gene. A typical value is 100 bp (for *Arabidopsis thaliana*).
- the `Riken.Max_overlap` parameter controls how information on "overlapping" regions is handled. If two EST pairs define transcribed regions with a large overlap (larger than the parameter value), then it is likely that they refer to the same gene (as far as they are detected as being on the same strand). In this case, the two EST pairs are taken as one (merged by taking the leftmost extremity as the new left extremity and the rightmost as the right extremity). If the two overlapping regions are not on the same strand, then they are considered as inconsistent and the orientation is forgotten.

  If the two regions have a small overlap (lower than the parameter value), then it is likely that there are 2 different genes with overlapping UTR. Because EUGÈNE cannot predict overlapping UTR, then the extremities are modified so that they do not overlap anymore.

Integration of information Basically, when a genomic region is validated, the plugin forces EUGÈNE to predict one single gene in the region. This is done by penalizing all tracks but the intergenic track just before and after the gene extremities and by penalizing the intergenic track on the genomic region itself. If the strand is also considered as detected, then all tracks on the other strand are also penalized. Although an infinite (eg. `-1e999` in the current double format) penalty would seem more appropriate, we advocate for a strong finite penalty to avoid stupid uselss predictions in case of data inconsistency.

Post analyse No post analyze.

Graph The Riken information is plotted on the output graph as two small corners delimiting the region on the intergenic track. The corners are colored differently according to the strand detected for the transcribed region.

1.5.5.4 `Sensor.NcRNA`

Description The plugin allows to take into account information from non protein coding RNA. For each reading ncRNA region, it rewards the ncRNA tracks, and the ncRNA transcription start and stop signals at the region extemities.

To activate the sensor, put the number of NcRNA instances you want to create to the parameter `Sensor.NcRNA.use` in the parameter file.

Here is an example of NcRNA parameters definition:

```
NcRNA.FileExtension[0]  ncrna
NcRNA.NpcRna*[0]        1
NcRNA.TStartNpc*[0]     1
NcRNA.TStopNpc*[0]      1
NcRNA.format[0]         GFF3 # Mandatory

Sensor.NcRNA.use        1
Sensor.NcRNA            1
```

Gff3 input files format The gff3 input mode is activated by setting the value `GFF3` for the parameter `NcRNA.format` in the parameter file. Note that for the moment, it is mandatory because gff3 is the only reading input format. The plugin reads its information from the file whose name is the concatenation of the sequence file name, the `NcRNA.FileExtension` parameter and the `.gff3` extension. The accepted features (third column) are:

- SO:0000655 or ncRNA
- all the terms derived from SO:0000655. For instance, SO:0000252 or rRNA, SO:0000253 or tRNA. For details, see `http://www.sequenceontology.org/wiki/index.php/Category:SO:0000655_!_ncRNA`.

If the feature used isn't one of those, the line will be rejected.

Here an extract of `seq14ac002535g4g5.tfa.ncrna.gff3`:

```
seq14       tRNAscan-SE      tRNA       4809       6126       .       +       .       ID=trna:seq14.5;
seq14       tRNAscan-SE      tRNA       7000       7100       .       +       .       ID=trna:seq14.5;
```

Filtering input information No filtering.

Integration of information At each position of a ncRNA region, the ncRNA content edge is reweighted according to the `NcRNA.NpcRna*` parameter value. ncRNA transcription start and stop signals are generated at each respective extremity according to the `NcRNA.TStartNpc*` and `NcRNA.TStopNpc*` parameter values.

Post analyse No post analyse.

Graph No plotting.

1.5.6 `Others plugins`

1.5.6.1 `Sensor.GCPlot`

Description The GCPlot sensor allows to add to the graphical representation a plot of basic composition statistics on the sequence. The sensor is activated by setting the parameter `Sensor.GCPlot.use` to `1` in the parameter file. The composition statistics represented can be arbitrarily chosen. For example, the GC%=$\frac{G+C}{A+T+G+C}$ is selected by setting `GCPlot.Up` to `GC` and `GCPlot.Over` to `ATGC`. Statistics on the 3rd base of each codon are automatically computed and plotted.

The color (integer between 0 and 8), the smoothing window width and specific zooming factors can be given. The zooming factor for the 3rd base in each codon is zoomed using specific zooming factor `GCPlot.Zoom3`

Here is an example of a GCPlot parameter definition :

```
GCPlot.Up          GC
GCPlot.Over        ATGC
GCPlot.Smooth      98
GCPlot.Color       5     # light green
GCPlot.Zoom        2.0
GCPlot.Zoom3       1.0
Sensor.GCPlot.use  1  # use GCPlot sensor
Sensor.GCPlot      1     # sensor priority
```

Input files format No input file.

Integration of information This sensor does not influence prediction.

Post analyse No post analyse.

Graph The composition statistics is plotted on the intergenic (IG) track. The same statistics computed on the 3rd position of each codon is plotted on the 6 exonic tracks.

1.5.6.2 `Sensor.GFF`

Description The GFF sensor allows to add to the graphical representation an annotation provided in a GFF format. Note that the provided GFF annotation could be an EuGÈNEprediction given in GFF format (obtained using the $-pg$ argument). This could allow to visualise two predictions on the same graph.

For a sequence, the plugin reads the annotation from one file whose name is derived from the sequence name by adding the `.gff` suffix. The sensor is activated by either :

- the `-G` argument
- the value 1 for the parameter `Sensor.GFF.use` in the parameter file.

Here is an example of GFF parameters definition :

```
Sensor.GFF.use  1      # Use GFF sensor
Sensor.GFF      1         # Sensor priority
```

Native input files format The file `.gff` describes an annotation for a sequence. The format of a line is : `<seqname> <source> <feature> <start> <end> <score> <strand> <frame>`. Seqname, source and score fields are ignored.

Example:

```
seqName  EuGene  Utr5   1      199    0      -      .
seqName  EuGene  Utr5   340    359    0      +      .
seqName  EuGene  Init   360    393    0      +      2
seqName  EuGene  Intr   596    732    0      +      0
seqName  EuGene  Intr   830    876    0      +      1
seqName  EuGene  Intr   961    1286   0      +      1
seqName  EuGene  Intr   1396   1478   0      +      2
seqName  EuGene  Intr   1573   1648   0      +      0
seqName  EuGene  Intr   1757   1818   0      +      0
seqName  EuGene  Intr   1962   2057   0      +      2
seqName  EuGene  Intr   2145   2306   0      +      2
seqName  EuGene  Term   2491   2607   0      +      0
seqName  EuGene  Utr3   2608   2626   0      +      .
```

Note: only exons are plotted, this file is parsing by the frame field (no '.' in the frame field).

Gff3 input files format The gff3 input mode is activated by setting the value `GFF3` for the parameter `GFF.format` in the parameter file. The plugin reads the predictions from a file which name is derived from the sequence name by adding the `.gff.gff3`

Accepted features (third column of GFF3 lines):

- SO:0000316 or CDS
- SO:0000204 or five_prime_UTR
- SO:0000205 or three_prime_UTR

If the feature isn't one of those, the line won't be take into account. If you define parent link between feature , parent feature must be define before children. Here an extract of `seq14ac002535g4g5.tfa.gff.gff3`.

```
seq25   EuGene  five_prime_UTR 1        2787    0       +       .       ID=five_prime_UTR:seq25.0;Ontology_term=SO:0000204
seq25   EuGene  CDS     2788    2836    0       +       0       ID=CDS:seq25.1;Ontology_term=SO:0000196
seq25   EuGene  CDS     8356    8471    0       +       2       ID=CDS:seq25.2;Ontology_term=SO:0000004
seq25   EuGene  CDS     8576    8667    0       +       1       ID=CDS:seq25.3;Ontology_term=SO:0000004
seq25   EuGene  CDS     9006    9061    0       +       0       ID=CDS:seq25.4;Ontology_term=SO:0000004
seq25   EuGene  CDS     9567    9655    0       +       1       ID=CDS:seq25.5;Ontology_term=SO:0000004
seq25   EuGene  CDS     10520   10535   0       +       1       ID=CDS:seq25.6;Ontology_term=SO:0000004
seq25   EuGene  CDS     10896   11134   0       +       1       ID=CDS:seq25.7;Ontology_term=SO:0000004
seq25   EuGene  CDS     11544   12005   0       +       2       ID=CDS:seq25.8;Ontology_term=SO:0000004
seq25   EuGene  CDS     12088   12900   0       +       0       ID=CDS:seq25.9;Ontology_term=SO:0000197
seq25   EuGene  three_prime_UTR 12901   14900   0       +       .       ID=three_prime_UTR:seq25.10;Ontology_term=SO:0000205
```

Filtering input information No filter.

Integration of information This sensor does not affect prediction.

Post analyse No post analyse.

Graph Orange horizontal lines are plotted on the exon tracks.

Documentation of the Plotter sensor

1.5.6.3 `Sensor.Plotter`

Description The Plotter sensor allows to add to the graphical representation the GC%, the GC3% and the two quotients A/T+A and T/T+A.

The sensor is activated by the value 1 for the parameter `Sensor.Plotter.use` in the parameter file.

Here is an example of Plotter parameters definition :

```
Plotter.GC          1         #
Plotter.GC3         1         # 0 -> no plot  -  1 -> plot
Plotter.A|T/A+T     1         #
Sensor.Plotter.use  1      # Use GFF sensor
Sensor.Plotter      1         # Sensor priority
```

Input files format No input files needed.

Integration of information This sensor does not affect prediction.

Post analyse No post analyse.

Graph The GC% is plotted as a thin turquoise line on the intergenic track. The GC3% is plotted as a thin turquoise line on each exon tracks. The T/T+A quotient is plotted as a thin orange line on the forward intron track. The A/T+A quotient is plotted as a thin orange line on the reverse intron track.

1.5.6.4 `Sensor.Tester`

Description The Tester sensor allows to evaluate signal sensors. For a sequence, the plugin reads the truth gene coordinates (note only one complete gene) in GFF format from one file whose name is derived from the sequence name by adding the `.gff` suffix.

Depending of the value of the parameter `Tester.Make`, two independant tests could be done. If `Tester.Make` is set to TEST, the positive positions (where the sensor detects a signal) are analysed (compared to the thruth). The results are written in a file `test.<sensorName.gff>`.

If the parameter `Tester.Make` is set to SPSN, the positions of the canonical coding of the considered signal (ATG for Start; TAG, TAA, TGA for Stop; AG for acceptor; GT, GC for donors) are analysed. The considering signal is defined following the value given at the `Tester.SPSN.Eval` parameter. Four variables are computed:

- TP, number of True Positive
- FN, number of False Negative
- FP, number of False Positive
- TN, number of True Negative

With these variables, two others are evaluated:

- Sn = TP/(TP+FN), sensitivity
- Sp = TP/(TP+FP), specificity

All these variables are computed for all score value given by the sensor. Each value is in turn considered as a threshold (if the score is higher than the threshold the information is considered as positive).
The values of the variables are put on stdout. Here is an example.

```
Thres.          Nb       TP       FP       TN       FN       Sens.           Spec.
-3.7297         1        144      16776    230      0        0.851064        100
-3.68888        1        144      16775    231      0        0.851114        100
-3.61192        1        144      16774    232      0        0.851164        100
-3.57555        1        144      16773    233      0        0.851215        100
[...]
```

Where Thres. is the threshold value, Nb is the number of observation of the threshold as a score.

To be plotted, specificity and sensibility are also written in the file `Sensor.<sensorName>.SpSn` and if the `Tester.SPSN.Eval` parameter is set to SPLICE, in the files `Sensor.<sensorName>.Acc` (for acceptor only), `Sensor.<sensorName>.Don` (for donor only).
Note that specificity and sensitivity are written in the `.SpSn`, `.Acc`, `.Don` files, only if TP+FP and TP+FN are higher than `Tester.SPSN.MinNumbers`. This to avoid Sp and Sn based on small effective.

The sensor is activated by the value 1 for the parameter `Sensor.Tester.use`. A parameter `Tester.Sensor` indicates which sensor to test. An other parameter `Tester.Sensor.Instance` defines wich instance (see the 2.2.1 Loading plugins section) of sensor to consider.

Here is an example of Tester parameters definition:

```
Tester.Make              SPSN       # SPSN, TEST
Tester.Sensor            EuStop
Tester.Sensor.Instance   0
Tester.SPSN.MinNumbers   100        # greater than 0
Tester.SPSN.Eval         STOP       # START, STOP, SPLICE
Sensor.Tester.use        1          # use Tester sensor
Sensor.Tester            1          # sensor priority
```

Native input files format The file `.gff` describes the truth coordinates of only one complete gene in GFF format. The format of a line is :`<seqname> <source> <feature> <start> <end> <score> <strand> <frame>`. Seqname, source, score and frame fields are ignored.

Example:

```
seqName EuGene  UTR5    866     885     0       +       .
seqName EuGene  E.Init  886     931     0       +       0
seqName EuGene  E.Intr  1014    2366    0       +       1
seqName EuGene  E.Term  2444    2481    0       +       1
seqName EuGene  UTR3    2482    2632    0       +       .
```

Note : Feature field must be UTR5, UTR3, E.Init, E.Intr, E.Term or E.Sngl (UTR states are optional).

Gff3 input files format The gff3 input mode is activated by setting the value `GFF3` for the parameter `Tester.format` in the parameter file. The plugin reads the predictions from a file named from the sequence name by adding the `.gff.gff3` extension.

Accepted features (third column):

- SO:0000316 or CDS

- SO:0000204 or five_prime_UTR
- SO:0000205 or three_prime_UTR

If the feature used isn't one of those, the line will be rejected. You must define the ontology_term for CDS features in order to identify the different types of exons (E.Init, E.Intr, E.Term, E.Sngl). Here is the matching between Eugene native feature name and Sofa feature:

- UTR5 : SO:0000204 (five_prime_UTR) ; Ontology term : not necessary
- UTR3 : SO:0000205 (three_prime_UTR); Ontology term : not necessary
- E.Init : SO:0000316 (CDS) ; Ontology term : SO:0000196 (five_prime_coding_exon_region)
- E.Intr : SO:0000316 (CDS) ; Ontology term : SO:0000004 (interior_coding_exon)
- E.Term : SO:0000316 (CDS) ; Ontology term : SO:0000197 (three_prime_coding_exon_region)
- E.Sngl : SO:0000316 (CDS) ; Ontology term : SO:0005845 (single_exon)

Gff3 attributes specifications:
Required attributes:

- ID
- Ontology_term (Required for CDS feature)

Here an extract of `seq14ac002535g4g5.tfa.gff.gff3`:

```
seq25   EuGene  five_prime_UTR  1       2787    0       +       .       ID=five_prime_UTR:seq25.0;Ontology_term=SO:0000204
seq25   EuGene  CDS     2788    2836    0       +       0       ID=CDS:seq25.1;Ontology_term=SO:0000196
seq25   EuGene  CDS     8356    8471    0       +       2       ID=CDS:seq25.2;Ontology_term=SO:0000004
seq25   EuGene  CDS     8576    8667    0       +       1       ID=CDS:seq25.3;Ontology_term=SO:0000004
seq25   EuGene  CDS     9006    9061    0       +       0       ID=CDS:seq25.4;Ontology_term=SO:0000004
seq25   EuGene  CDS     9567    9655    0       +       1       ID=CDS:seq25.5;Ontology_term=SO:0000004
seq25   EuGene  CDS     10520   10535   0       +       1       ID=CDS:seq25.6;Ontology_term=SO:0000004
seq25   EuGene  CDS     10896   11134   0       +       1       ID=CDS:seq25.7;Ontology_term=SO:0000004
seq25   EuGene  CDS     11544   12005   0       +       2       ID=CDS:seq25.8;Ontology_term=SO:0000004
seq25   EuGene  CDS     12088   12900   0       +       0       ID=CDS:seq25.9;Ontology_term=SO:0000197
seq25   EuGene  three_prime_UTR 12901   14900   0       +       .       ID=three_prime_UTR:seq25.10;Ontology_term=SO:0000205
```

Output files format For the parameter `Tester.Make` set to TEST, a file (`test.<sensorName.gff>`) is created if it does not exist. For each predicted signals the Tester sensor write one line in the output file. The format of this line is : `<seqname> <source> <feature> <score> <start> <end> <strand> <frame> <T/F> <state>`.

Where:

- <seqname> is the 7 first characters of the sequence file name.
- <source> is the name of the tested sensor.
- <feature> is the feature type name (can be 'Start', 'Acc', 'Don' and 'Stop').
- <start> is the predicted signal position.
- <end> is always '.'.
- <score> is the score given by the tested sensor.
- <strand> is '+' for forward and '-' for reverse.
- <frame> is always '.'.

- <T/F> is ‘True’ for real site and ‘False’ for the others.
- <state> is the real state according to the predicted signal position (can be ‘IG’, ‘UTR’, ‘ExonF’, ‘ExonR’, ‘IntronF’ or ‘IntronR’).

Here is an extract of `test.NG2.gff` :

```
[...]
seqName     NG2     Acc      835       .   -2.26       -       .   False       IG
seqName     NG2     Acc      869       .  -15.03       +       .   False      UTR
seqName     NG2     Acc      918       .  -10.96       -       .   False    ExonF
seqName     NG2     Don      931       .   -0.02       +       .    True    ExonF
seqName     NG2     Don      962       .  -33.06       -       .   False  IntronF
seqName     NG2     Don      973       .  -27.76       +       .   False  IntronF
seqName     NG2     Don     1011       .   -4.10       -       .   False  IntronF
seqName     NG2     Acc     1013       .   -0.10       +       .    True  IntronF
seqName     NG2     Acc     1050       .   -7.52       +       .   False    ExonF
seqName     NG2     Don     1050       .  -27.76       +       .   False    ExonF
[...]
```

For the parameter `Tester.Make` set to SPSN, the file `Sensor.<sensorName>.SpSn` contains on each line a value of specificity and sensitivity for a threshold taken from the lowest to the highest.
For splice detectors sensors, two other files are also written `Sensor.<sensorName>.Acc` (acceptor only), `Sensor.<sensorName>.Don` (donor only) with the same format.

Filtering input information No filter.

Integration of information This sensor does not affect prediction.

Post analyse No post analyse.

Graph No plot.

1.6 EUGÈNE as a combiner

EUGÈNE is able to integrate predictions from many sources and to combine them in one prediction. For that, you only need to use the AnnotaStruct plugin (see section 1.5.5.1): create as many AnnotaStruct instances as files to combine.

The parameter file `EUGENEDIR/cfg/eugene.combine.par` is parametrized to combine two files. In the first file, information about start and stop codons is taken into account, whereas in the second one, it is information about splice sites and CDS.

```
 ##### Sensors AnnotaStruct #####
AnnotaStruct.FileExtension[0]      genefinder1
AnnotaStruct.TranscriptFeature[0]  transcript
AnnotaStruct.Start*[0]            2     # i: inline score (GFF3 format only)
AnnotaStruct.StartType[0]         s      # p: probability  s: score
AnnotaStruct.Stop*[0] 1.5
AnnotaStruct.StopType[0] s
AnnotaStruct.Acc*[0] 0
AnnotaStruct.AccType[0] s
AnnotaStruct.Don*[0] 0
AnnotaStruct.DonType[0] s
AnnotaStruct.TrStart*[0] 0
AnnotaStruct.TrStartType[0] s
AnnotaStruct.TrStop*[0] 0
AnnotaStruct.TrStopType[0] s
AnnotaStruct.TrStartNpc*[0] 0
AnnotaStruct.TrStartNpcType[0] s
AnnotaStruct.TrStopNpc*[0] 0
AnnotaStruct.TrStopNpcType[0] s
AnnotaStruct.Exon*[0] 0
AnnotaStruct.Intron*[0] 0
AnnotaStruct.CDS*[0] 0
AnnotaStruct.npcRNA*[0]  0
AnnotaStruct.Intergenic*[0]  0
AnnotaStruct.format[0]             GFF3

AnnotaStruct.FileExtension[1]      genefinder2
AnnotaStruct.TranscriptFeature[1]  transcript
AnnotaStruct.Start*[1]            0     # i: inline score (GFF3 format only)
AnnotaStruct.StartType[1]          s      # p: probability  s: score
AnnotaStruct.Stop*[1] 0
AnnotaStruct.StopType[1] s
AnnotaStruct.Acc*[1] 3
AnnotaStruct.AccType[1] s
AnnotaStruct.Don*[1] 2.5
AnnotaStruct.DonType[1] s
AnnotaStruct.TrStart*[1] 0
AnnotaStruct.TrStartType[1] s
AnnotaStruct.TrStop*[1] 0
AnnotaStruct.TrStopType[1] s
AnnotaStruct.TrStartNpc*[1] 0
AnnotaStruct.TrStartNpcType[1] s
AnnotaStruct.TrStopNpc*[1] 0
AnnotaStruct.TrStopNpcType[1] s
AnnotaStruct.Exon*[1] 0
AnnotaStruct.Intron*[1] 0
AnnotaStruct.CDS*[1] 4
AnnotaStruct.npcRNA*[1]  0
AnnotaStruct.Intergenic*[0]  0
AnnotaStruct.format[1]             GFF3
#
# SIGNAL/CONTENT SENSORS
Sensor.AnnotaStruct.use 2
#
```

More details about AnnotaStruct parameters in the section 1.5.5.1.

1.7 Optimization of Plugins parameters

The value of some numerical plugins parameters (specified in the parameter file with a name finishing with an '*') can be optimized on a reference set of sequences (with their related information) for which genes positions are known. The idea is to adapt the values of parameters to increase as much as possible the quality of prediction of genes and exons. The figure 1.1 details the general function of the software with input and ouput files.

The optimization can be lauched with the `-Z` argument on the command line or with the `ParaOptimization.Use` parameter set to `1`.

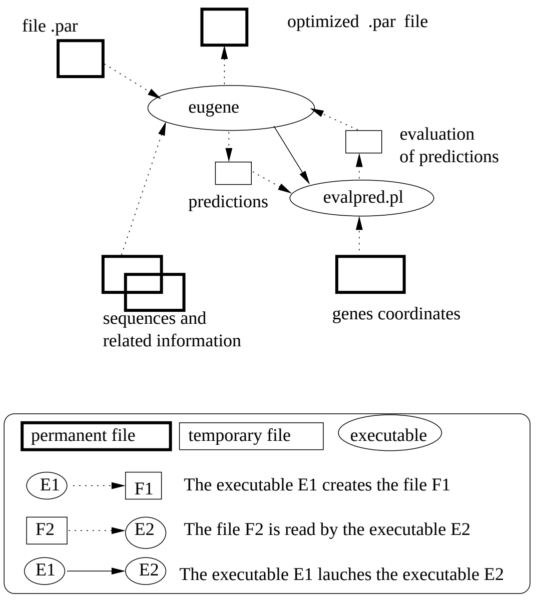

file .par
optimized .par file
eugene
evaluation of predictions
predictions
evalpred.pl
sequences and related information
genes coordinates
permanent file
temporary file
executable
E1
F1
The executable E1 creates the file F1
F2
E2
The file F2 is read by the executable E2
E1
E2
The executable E1 lauches the executable E2

Figure 1.1: Input and output files for parameters optimization

After updating the parameter file `eugene.par` (which sensors to use,...), the software is lauched with the usual command line specifying as argument the reference sequences to consider. At the end, the software creates a new parameter file called `eugene.<date>.OPTI.par` (for example, `eugene.30Sep-2003.OPTI.par`) with the new value for the optimized parameters.

For parameters optimization, the inputs to be specified in the parameter file are:

- the parameters to optimize with their value domain,
- the optimization algorithm to use: genetic algorithm, Line Search, genetic algorithm and Line Search,
- the parameters of the optimization algorithm, and for the Line Search algorithm complementary information on the parameters to optimize (initial value, step of discretization, ...),
- a file with the coordinates of genes for the sequences set. See below its description.
- it is possible to include a regularizer term in the criteria optimized using the `ParaOptimization.Regularizer` parameter. The sum of all the absolute values of the parameters (L1-norm) multiplied by the value of this parameter is used to penalize the original fitness to define the final fiteness optimized.
- `Eval.offset`: during the evaluation of a prediction, the prediction is compared with a reference (the real gene coordinates). The region in which compare the prediction and the reference is defined as the reference positions +/- the offset.
- `Eval.ignoreNpcRNA`: Put 1 to ignore the npcRNA for the fitness computing.
- `Fitness.wsng`, `Fitness.wsne`, `Fitness.wsnn`, `Fitness.wspg`, `Fitness.wspe`, `Fitness.wsspn` : indicate respectively the weight of the gene sensitivity, of the exon sensitivity, of the nucleotide sensitivity, of the gene specificity, of the exon specificity and of the nucleotide specificity in the fitness computing.

Description of the file with the coordinates of genes One line of the file describes one gene. The first field of the line is the sequence name. The followed fields are the list of respectively start and stop positions of the exons of the gene. The fields are separated by spaces. An empty line is required to separate two different sequences. Note that the order of the sequences is important: the order has to be similar to the result of the 'ls 'command.

Example:

```
SEQ1 429 545 665 750
SEQ1 -2001 -2342 -2424 -2522

SEQ2 1000 1230 1521 1690 2510 2600
```

This example describes 2 sequences named SEQ1 et SEQ2. SEQ1 is composed of two genes: the first gene is composed of two exons on the forward strand [429-545] [665-750], the second of two exons on the reverse strand [2001-2342] [2424-2522]. SEQ2 has a unique gene composed of three exons [1000-1230] [1521-1690] [2510-2600]

Optimization parameter definition example Here is a simplified example of optimization parameters definition in the parameter file.

```
#################################################################
################### PARAMETERS OPTIMIZATION #####################
#################################################################
ParaOptimization.Use           1
ParaOptimization.Regularizer   0.0
ParaOptimization.TrueCoordFile Araset.coord
ParaOptimization.Algorithm     GENETIC+LINESEARCH
ParaOptimization.Test          FALSE
ParaOptimization.Trace         1
#
ParaOptimization.NbParameter   3
#
ParaOptimization.Para.Name[0]   NStart.startP*
ParaOptimization.Para.Min[0]    0.001
ParaOptimization.Para.Max[0]    15
#
ParaOptimization.Para.Name[1]   NStart.startB*
ParaOptimization.Para.Min[1]    0.001
ParaOptimization.Para.Max[1]    15
#
ParaOptimization.Para.Name[2]   EuStop.stopP*
ParaOptimization.Para.Min[2]    0
ParaOptimization.Para.Max[2]    6
#
################## Genetic ######################################
Genetic.NbRun 2
Genetic.NbGeneration 20
Genetic.NbElement 50
Genetic.Seed 4
Genetic.CrossOverProbability 0.6
Genetic.MutationProbability 0.2
Genetic.SelectionType 1         # 0: roulette wheel
#                                 1:stochastic remainder without replacement
Genetic.ScalingType 1           # 0: no scaling
#                                 1: Sigma Truncation scaling
#                                 2: Power Law scaling
Genetic.Sharing 0.9             # 0: no sharing
#                                 1: sharing, looking for clusters which best
#                                    elt fitness is at least n% of the overall
#                                    best element of the population
Genetic.Clustering 1
Genetic.Elitism 0.9             # 0: none
#                                 n: elitism; keeps the best elt if no sharing,
#                                    and keeps the best elt of each cluster
#                                    which best_elt fitness is at
#                                    least n% of the overallbest elt if sharing
Genetic.SA.Mutation FALSE          # Simulated Annealing mutation
Genetic.SA.CrossOver FALSE         # Simulated Annealing crossover
#
#
######### LINESEARCH ###########################################
```

```
LineSearch.NbMaxCycle 1
LineSearch.NbMinCycle 1
LineSearch.NbMaxStab 2
LineSearch.DivInter 10
LineSearch.Alpha 0.6
LineSearch.EvolutionMini 0.001
LineSearch.Seed ALEA
#
LineSearch.NbCluster 2
LineSearch.Cluster[0] LINKED
LineSearch.Cluster[1] IDENTICAL
#
LineSearch.Para.Step[0]         0.001
LineSearch.Para.Init[0]         7.5
LineSearch.Para.MinInit[0]      0.001
LineSearch.Para.MaxInit[0]      15
LineSearch.Para.Cluster[0]      0
#
LineSearch.Para.Step[1]         0.001
LineSearch.Para.Init[1]         7.5
LineSearch.Para.MinInit[1]      0.001
LineSearch.Para.MaxInit[1]      15
LineSearch.Para.Cluster[1]      0
#
LineSearch.Para.Step[2]         0.001
LineSearch.Para.Init[2]         3
LineSearch.Para.MinInit[2]      0
LineSearch.Para.MaxInit[2]      6
LineSearch.Para.Cluster[2]      1
#
```

1.8 Command line flags

- `a`: activates the alternative splicing prediction.
- `b`: activates the plugin `Sensor.BlastX`.
- `B`: postprocessing activation of the plugin `Sensor.BlastX`.
- `c`: controls how successives PNG images overlap (parameter `Output.golap`). It must be followed by the number of overlapping nucleotides between 2 successives PNG images. Default is heuristically determined based on resolution and number of nuc. per image.
- `d`: activates the plugin `Sensor.Est`.
- `D`: allows to specify a value to a parameter (syntax: -D<para>=<value>).
- `E`: enables EST and cDNA post-predition analysis (parameter `Est.PostProcess`) of the Est sensor: after each transcript prediction, all matching EST are analyzed and the consistency of the EST with the prediction is analyzed. At the end, the number of bases of the exon/intron structure predicted which are consistent with at least one EST/cDNA are reported.
- `f`: the frameshift penalty. A large value prevents EUGÈNE from predicting frameshifts (the default).
- `g`: graph required.
- `G`: activates the plugin `Sensor.GFF`.
- `h`: help
- `l`: controls the number of nucleotides that will appear on a single image (parameter `Output.glen`). Default is min (6,000 length to visualize). The length to visualize is computed from the value given to -u and -v (default is all sequence)
- `m`: activates the plugin `Sensor.MarkovIMM` and specifies the filename of the set of Markov models that will be used by the MarkovIMM sensor (parameter `MarkovIMM.matname`).

- `M`: activates the plugin `Sensor.MarkovProt` and specifies the filename of the set of Markov models that will be used by the MarkovProt sensor (parameter `MarkovProt.matname`).
- `n`: followed by 0 1 or 2. Indicates the way the score are normalized accross the possibles states (phase 1,2,3,-1,-2,-3, introns and intergenic states).
  - `0`: no normalization
  - `1`: normalize accross all states
  - `2`: normalize each coding phase w.r.t. to the non coding score only.

  Default is 1 (parameter `Output.normopt`). Does not affect prediction, only text/graphical output.

- `o`: allows to offset the nucleotide position of the prediction (parameter `Output.offset`). That is, the prediction for nucleotide at position i of the given sequence is printed as nucleotide $i+$ the offset. Useful to perform prediction on an extracted sequence without loosing the original position.
- `O`: allows to specify an output directory (the texttttOutput.Prefix parameter value).
- `p`: controls the format of the textual outpout (parameter `Output.format`). May be d (detailed), l (long), s (short), h (html), g (gff) or a (araset format). Default is l.
- `r`: activates the plugin `Sensor.Repeat`.
- `R`: activates the plugin `Sensor.Riken`.
- `s`: forces non partial gene mode prediction. This forbids predictions that start and end in intergenic mode and therefore prevents the occurrence of partial gene structures on the border of the sequence. Useful if EUGÈNE lacks context around the gene and you know a single (or only complete) gene appears on the sequence. In practice this simply sets the parameters `EuGene.ExonPrior`, `EuGene.IntronPrior`, `EuGene.FivePrimePrior` and `EuGene.ThreePrimePrior` to 0.0.
- `t`: activates the plugin `Sensor.Homology`
- `u`: controls the part of the sequence whose prediction will be displayed in the graphical output (parameter `Output.gfrom`). It must be followed by the position of the 1st nuc. which will be plotted on graphical output (allows for zoom'in). Default is 1.
- `U`: activates the User information sensor (parameter `Sensor.User.use`). This sensor reads user informations stored in .user file. These informations use a small language. The language can contain two types of statements. Statements on signals (translation start, splice sites) and on the sequence itself (coding, non coding...).
- `v`: controls the part of the sequence whose prediction will be displayed in the graphical output (parameter `Output.gfrom`). It must be followed by the position of the last nuc. which will be plotted on graphical output (allows for zoom'in). Default is the sequence length.
- `w`: followed by half the size of the smoothing window for the scores (parameter `Output.window`). Default is 48. Does not affect prediction, only graphical output.
- `x`: controls the horizontal resolution of the PNG images generated by EuGene (parameter `Output.resx`). Default is 900.
- `y`: controls vertical resolution of the PNG images generated by EuGene (parameter `Output.resy`). Default is 400.
- `Z`: allows to ask for a parameters optimization (equivallent to set the `ParaOptimization.Use` parameter to 1).

Index of command line flags for sensors

For more information, have a look to www-bia.inra.fr/T/EuGene. This gives a rough idea of EUGÈNE reliability and the meaning of the graphical output (PDF file, poster on EUGÈNE).