

JIGSAW README

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1 Preamble

This software is OSI Certified Open Source Software.

OSI Certified is a certification mark of the Open Source Initiative.

2 Introduction

JIGSAW is a software program designed to construct gene models from multiple sources of prediction evidence. It can take as input gene prediction programs, sequence alignment data and splice site prediction programs, which map to a supplied genomic sequence. The standard JIGSAW program is called "jigsaw" and is designed to operate on a single user supplied fasta formatted genomic sequence.

3 Getting Started

In order to get JIGSAW up and running you first have to decide what gene structure evidence to collect from your annotation pipeline. At a minimum it is expected that two (preferably three) different gene prediction programs are used and two different sets of alignments will be used (alignments from a protein and transcript database respectively). Although it may be possible to use less evidence it is not recommended. Additional supplemental evidence may also be useful including custom transcript databases, additional gene predictions programs and splice site prediction programs. Once the set of evidence sources is determined, JIGSAW can be used in one of two ways, with the linear combiner option or with the statistical combiner option.

The statistical combiner is trained to evaluate the accuracy of the different combinations of evidence. This is done by providing examples of known genes and comparing the output from your gene structure annotation pipeline with the structure of the known genes. This is also referred to as, "training JIGSAW". During the training process, JIGSAW builds statistical models of the expected combinations of evidence that correspond to different parts of the gene structure. These models are used to piece together new genes in previously unseen data.

The linear combiner option works by assigning a weight to each evidence source and piecing together gene models that maximize the weight sum of the

predicted evidence. The advantage of this approach is that weights can be assigned without relying on a training set. For example each evidence source can be assigned equal weight, or cDNA alignments can be assigned a higher weight than ab initio gene prediction sources.

The next section describes running JIGSAW's linear combiner option.

4 RUNNING JIGSAW

The script, "run_jigsaw.pl" is designed to run JIGSAW on multiple sequence contigs using a template evidence list file. It is assumed that there is a separate directory for each sequence contig and the gene prediction evidence associated with the contig, is located in that directory. "run_jigsaw.pl" run the "jigsaw" binary for each individual directory containing data.

The basic command line options for running JIGSAW (after training) are:

jigsaw -f "fasta seq file" -d "training directory" -m "gene output file" -e "evidence list file"

-f: Genomic sequence in fasta format associated with evidence

-d: directory where training data resides.

-m: outputs gene models to this file, in a simple gff format.

-e: A simple flat file that lists the locations and type of each piece of evidence.

Each line in the file must contain the information in the following order:

1) file name containing evidence

2) file format of the file (see: FEEDING DATA TO THE JIGSAW, for supported file formats)

3) the type of evidence ("geneprediction", "spliceprediction" or "homology")

4) types of predictions the evidence makes, accepts any combination of the following:

"acc" (acceptor), "don" (donor), "start", "stop", "intron" and "coding"

A third column is a space separated list of the types of predictions the evidence

makes, any combination of acc (acceptor), don (donor), start, stop, intron and coding

are allowed.

example:

```
/data/genepredictor1 default geneprediction acc donor coding start stop intron
```

```
/data/proteins1 default homology acc donor coding start stop
```

```
/data/splicepredictions default spliceprediction acc don
```

IMPORTANT

The evidence list order, must correspond to the evidence list order used in

training. This means that even if a particular directory does not have all of the evidence, the file where the evidence would be in, must be listed in the evidence file.

The same evidence file (with minor modifications) can be used for training and running.

The typical usage of the script is:

```
run_jigsaw.pl -l directory_file_list -e evidence_template_list -o output_filename  
-d training_directory
```

4.1 Running the linear combiner option

The linear combiner option is run nearly identical to the statistical combiner option with minor changes to the evidence list file format and option flags. When using the “run_jigsaw.pl” script, the “-lin” option is used and the -d option can be dropped. The jigsaw binary is called as follows:

```
jigsaw -l -f "fasta seq file" -m "gene output file" -e "evidence list file"
```

The evidence list file differs in format so that along with the listing of predictions made by each evidence source, is the weight associated with the prediction. Using the example used above for the statistical combiner option the difference is shown here:

```
/data/genepredictor1 default geneprediction acc 1.0 donor 1.0 coding 1.0  
start 1.0 stop 1.0 intron 1.0
```

```
/data/proteins1 default homology acc donor coding start 1.0 stop 1.0
```

```
/data/splicepredictions default spliceprediction acc 1.0 don 1.0
```

Note there is no preset range of weight values, except to assume all weights are greater than 0. However, there is an important assumption, which is that the weight is **multiplied** by the evidence source’s prediction score. Therefore, it is important that the evidence source’s prediction score be either set to “1” or you are careful with how the prediction score influences gene scores.

The next section describes the training process in more detail. In addition, there is a tutorial included with this distribution, which walks you through an example of training and running JIGSAW.

5 Training JIGSAW

The perl script “bin/train_jigsaw.pl” provides a template for how to train JIGSAW, and assumes there are multiple directories, each with a single contig and evidence associated with that contig. The script uses the library stored in the lib directory, “oc1” (uses the “mktree” binary). The script assumes that the

"mktree" binary is somewhere in your path. An important component of training and running JIGSAW is the creation of an evidence list file which lists each type of output generated by the annotation pipeline, and specifies the filenames containing the output of the gene structure annotation pipeline. The evidence list file must list each piece of evidence in the exact same order for both training and running. In other words if you are using two gene prediction files "gp1.txt" and "gp2.txt", if the evidence list file has an order like

- "gp1.txt default geneprediction acc don coding start stop intron"
- "gp2.txt default geneprediction acc don coding start stop intron"

then the listed order when running JIGSAW must match the order used in training. The format for the evidence list file is shown when you type "jigsaw" at the command line. The difference between the training evidence list file and the running evidence list file is there is an additional line in the training evidence list file, containing the filename of the true genes for training followed by file format and the string id, "curation".

ie.:

```
/data/answer.genes.txt default curation
```

The default file format for the "true" genes is equivalent to the default format for "geneprediction". See the section "FEEDING DATA TO JIGSAW" below. Training must take place in an area that can be "written" to, as new files are created (some temporarily). Running the "train_jigsaw.pl" will create a directory with a user specified name containing decision trees, and the example evidence vectors, plus a parameter file, "param.txt", containing some potentially useful tuning parameters. The decision tree construction can take a long time to run. To speed things up use the "-a" option to the "mktree" binary, which uses single condition splits for nodes in the tree. When using the "train_jigsaw.pl" script -t turns on the -a option for mktree.

6 Feeding Data To JIGSAW

Input File Formats

JIGSAW reads several file formats: "default", "btab", "gff", "glimmerm" and "phat", "fgenesH", "genemarkhmm", "genscan", "snap". Some formats are designed to read the output from specific programs, GlimmerM/GlimmerHMM, PHAT, FgenesH, Snap, Genemark and Genscan. These formats are compatible with the "geneprediction" evidence type. "jigsaw -S" lists the formats available for the latest version.

"btab" - is a tab delimited format which can be used to read "homology" data, only.

"gff" - (<http://www.sanger.ac.uk/Software/formats/GFF/>) is a more general format to specify sequence features, and can be used with any of the types "spliceprediction", "homology" and "geneprediction". Using "gff" for "geneprediction" type predictions requires that the feature description describe exons in some format that distinguishes "Initial", "Internal", "Single" and "Terminal" exons. Any strings with the following will do: "final", "start", "stop", "end", "begin", "first", "last". If you have a program that only predicts start or stop codons, use the "geneprediction" type and gff format with a feature description of "start" and "stop".

Because data may come from any number of different sources, the hope is, it will be relatively easy for users to write their own perl scripts to parse and convert additional data formats if need be to one of the simple formats used by the JIGSAW.

"default" -

Below is a description of the "default" formats for the three expected prediction types:

1) Gene Prediction Program (string identifier is "geneprediction")

Two different formats, use whichever is easier....

"gene model #" 5' 3' score

example:

45 219690 220936 1.0

or

"gene model #" "exon model type" 5' 3' score

example:

1 single 7623 7958 1.0

"exon model type" is: internal, single, terminal or initial

2) Homology Data (string identifier is "homology")

5' 3' r5' r3' "STRING ID" "% IDENTITY" "% SIMILARITY"

example:

8853 8806 256 271 GP|7290595|gb|AAF46045.1||AE003434 18.750000 37.500000

r5' and r3' refers to the relative alignments of the homologous sequence

r5' and r3' are used only for reference

3) Splice Site Prediction (GeneSplicer format) (string identifier is "spliceprediction")

5' 3' type score

where type is either "acceptor" or "donor"

example:

31906 31905 donor 1.0

IMPORTANT TIP:

With alignment data be sure to assign unique alignment identifiers. When a transcript is aligned to the sequence in multiple locations, it is important to distinguish each match uniquely in order for JIGSAW to separate out the predicted introns.

7 Using the Parameter File

The first time a jigsaw is run using a training directory, the program first checks to see if the training directory contains a “param.txt” file, if the file does not exist, it creates one using a default set of values. Contents of the default file are shown below.

```
# Intron Length Penalty
-1 10
# Intergenic Length Penalty
0 0
# Internal Exon Length Penalty
0 0
# Minimum Intron Length
10
# Minimum Intergenic Length
20
# Alignment Connection Cutoff
-1
# Donor Consensus Sequence
gt gc
# Acceptor Consensus Sequence
ag
# Maximum Sequence Length / Overlap Length
2000000 20000
```

- The values for a given option are preceded by a comment describing what the option is.
- # Intron Length Penalty = -1 10. The first parameter sets the threshold length which triggers the weight factor (the second parameter). The default -1, means this option is turned off. Length penalties can also be used for Intergenic and Internal Exon intervals.
- The user can require JIGSAW predictions of intergenic sequence length to exceed a minimum value, the default minimum is 20 bases.
- The Alignment Connection Cutoff by default (-1) is turned off. When the value is set to a positive integer N, alignments with the same transcript id are assumed to predict a single transcript. In general each alignment matching to the genomic sequence should be assigned a unique alignment identifier, to distinguish a transcript that maps to multiple genomic locations. However, when this information is not provided, the alignments that

have been assigned a single “transcript identifier” and are in close proximity (defined by N bases) are assumed to be part of the single predicted transcript.

- The donor census splice sites are defined by the user, the default is to allow both gt and gc splice sites to occur (and ag for acceptor sites).
- For long sequences, it will be faster for JIGSAW to run on overlapping subsequences and merge the predictions. The user can define what a “long” sequence is, and what the overlap size should be. The default is to use 2 million base sequence windows, with each window overlapping by 20,000 bases.

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