jFuzzyMachine – An Open-source Fuzzy Logic–based Regulatory Inference Engine for High–throughput Biological Data

Paul Aiyetan*1

1George Mason University, School of Systems Biology, Manassas, VA 20110

October 7, 2020

Abstract

Elucidating mechanistic relationships between and among intracellular macromolecules is fundamental to understanding the molecular basis of normal and diseased processes. Here, we introduce jFuzzyMachine – a fuzzy logic-based regulatory network inference engine for high-throughput biological data. We describe its design and implementation. We demonstrate its functions on a sampled expression profile of the vorinostat-resistant HCT116 cell line. We compared jFuzzyMachine’s inferred regulatory network to that inferred by the ARACNe (an Algorithm for the Reconstruction of Gene Regulatory Networks) tool. Potentially more sensitive, jFuzzyMachine showed a slight increase in identified regulatory edges

*paiyetan@gmu.edu
compared to ARACNe. A significant overlap was also observed in the identified edges between the two inference methods. Over 70 percent of edges identified by ARACNe were identified by jFuzzyMachine. Beyond identifying edges, jFuzzyMachine shows direction of interactions, including bidirectional interactions – specifying regulatory inputs and outputs of inferred relationships. jFuzzyMachine addresses an apparent lack of freely available community tool implementing a fuzzy logic regulatory network inference method – mitigating a limitation to applying and extending benefits of the fuzzy inference system to understanding biological data. jFuzzyMachine’s source codes and precompiled binaries are freely available at the Github repository locations:

https://github.com/paiyetan/jfuzzymachine and
https://github.com/paiyetan/jfuzzymachine/releases/tag/v1.7.21.

1 BACKGROUND

Elucidating mechanistic relationships among intracellular macromolecules is fundamental to understanding the molecular basis of normal and diseased processes. Traditional approaches to elucidating these involved low-throughput methods of obtaining reaction kinetics with attending resolution of associated partial or ordinary differential equations (ODEs) [6] [3] [5,21,42]. However, such approaches being highly sensitive to quantitative errors and increased complexities when the inferential problem involves multiple parameters are subject to inaccurate analytical estimates, with respect to experimental data from high-throughput profiling [14,37]. Partly to address these, the advent of high-throughput profiling approaches has been greeted with the development of reverse engineering and computational analyses methods that attempt to tease mechanistic relationships from attendant high-throughput data [6,11,45][13,35]. Amongst these, the classical fuzzy logic approach employs varying degrees of
truth to describe relationships between interacting molecules. The fuzzy logic reasoning methods [48], having had significant successes in the design and implementation of high-end control systems [47,49,50], were first applied to biological data by Woolf and Wang [46]. In the subsequent years, methods have been proposed to address identified shortfalls associated with the original implementation and to improve the inferential capability of the fuzzy approach [?,?4,7,12,16,24,25,31–34,43,44][38][36][9,15]. In spite of advances in the theoretical basis and relative biological validity of the fuzzy approach, there exists the very apparent lack of analytical tools [1] that implement these methods available to the scientific and research community. The apparent lack of ready and freely available community tools limits applying the fuzzy inference approach to biological data. This also limits necessary comparisons and bench-marking of results obtained by the method against those obtained from comparable methods. To elucidate mechanistic relationships, and to make more readily available the Fuzzy logic inference approach, we developed the jFuzzyMachine as a freely available tool.

2 MATERIALS AND METHODS

2.1 Dataset

To demonstrate jFuzzyMachine’s functionalities, including comparing results with similar computational tool and to show how jFuzzyMachine may be used to infer regulatory network interactions among a population of genes, we downloaded and derived a sampled expression profile from the NCBI GEO Dataset GSE56788. The dataset is detailed under the BioProject accession PRJNA244587. This consists of a total of 45 assay samples from 15 biosamples, each ran in 3 independent biological replicates. RNA-seq expression profiles were acquired.
by next-generation sequencing of vorinostat-resistant HCT116 cells, following knockdown of potential vorinostat-resistant candidate genes. Expression profiles were compared to mock transfection (control). The authors of the study sought to understand the mechanisms by which these knockdowns contributed to vorinostat response. They employed the siRNA-mediated knockdown of each of some previously identified resistance candidate genes in the HCT116-VR cell line. RNA sequence expression data were downloaded from the NCBI Sequence Read Archive [19,20], with accession number SRP041162.

2.1.1 RNA Sequence Analyses – quality assessment, preprocessing and normalization

For quality assessment (QA) of curated fastq files, the fastqcr, ngsReports and Rqc R/bioconductor tools [17] [10,28,30], modeled after the FASTQC [2] tool philosophy were used. To quantify expression, sequence reads were aligned to the genome (NCBI GRCh38 build) using the TopHat2 [18] [40,41] tool. Index files were downloaded from Illumina iGenomes archive. Accepted hits and annotation information in the BAM format [39] output files were assembled into an expression matrix of feature counts using the featureCount routine in the Rsubread package [22]. Feature counts were normalized using the DESeq2 package [23] tools-implemented regularized-log transformation to account for disparate total read counts in the different files and to allow for comparison across the different samples. For demonstration and evaluation purpose, the expression profile of 14 features were extracted. These include those for the genes PTHLH, KRT86, RUBCNL, CYS1, LINC00707, LINC00634, LINC00886, GCNT4, TAGLN3, ROBO4, MGAM, 2orf78, LOC105371789, and SERPINB7.
2.2 The fuzzy Logic Inference

Given an expression profile, the fuzzy logic approach involves three major steps:

- Fuzzification
- Rule evaluation, and
- Defuzzification

The fuzzification step derives qualitative values from the expression profile’s crisp values. It is often described as a mapping of non-fuzzy inputs to fuzzy linguistic terms [29]. Given qualitative values of HIGH, MEDIUM, or LOW, the fuzzification step takes a feature’s expression value and assigns it degrees to which it belongs to the respective class of HIGH, MEDIUM or LOW expression values. The rule evaluation step takes combinations of features and utilizes an inference engine rules of the form IF-THEN, including fuzzy set operations such as AND, OR, or NOT to evaluate input features in relation to outputs features. This has been described as attempting to make an expert judgment of collective linguistic terms; attempts to find a solution to an evaluation of the concurrent state of existence of linguistic description of states. The defuzzification step attempts to report a corresponding continuous numerical variable from a fuzzy state linguistic variable.

**jFuzzyMachine** implements the fuzzy inference steps described in [38][36][9, 15]. After an initial data transformation of \( \log_2 \) expression ratios by the arctan function and dividing values by \( \frac{\pi}{2} \), to project the ratios onto \([-1, 1]\), the fuzzification step utilizes a membership function consisting of three fuzzy sets (low, medium, and high expression). Given three fuzzy sets \( y_1 = \text{low}, y_2 = \text{medium}, y_3 = \text{high} \), its fuzzification of a gene expression value \( x \) results in the generation of a fuzzy set \( y = [y_1, y_2, y_3] \) as follows:
\[ y_1 = \begin{cases} x, & x < 0 \\ 0, & x \geq 0 \end{cases} \]
\[ y_2 = 1 - |x|, \forall x \]
\[ y_3 = \begin{cases} 0, & x \leq 0 \\ x, & x > 0 \end{cases} \]

We specified our rule configuration (the specification of if-then relationships between variables in fuzzy space) in the form of a vector \( r = [r_1, r_2, r_3] \). We specified the state of an output node \( z = [z_1, z_2, z_3] \) to be determined by the fuzzy state of an input feature \( y = [y_1, y_2, y_3] \) and the rule describing the relationship between the input and the output, \( r = [r_1, r_2, r_3] \) as follows:

\[ z = [y_{r1}, y_{r2}, y_{r3}] \]

An inhibitory relationship, for example, specified as \( [3, 2, 1] \) implies, if input is low \( (r_1) \), then output is high \( (3) \); if input is medium \( (r_2) \), then output is medium \( (2) \), and if input is high \( (r_3) \), then output in low \( (1) \). Classic fuzzy logic rule evaluation using the logical AND connective results in a combinatorial rule explosion i.e. an exponential increase in the number of rules to be evaluated and computational time, with additional inputs to be considered \([8]\). To address a combinatorial rule explosion situation, jFuzzyMachine implements the logical OR (union) rule configuration, an algebraic sum in fuzzy logic \([48][27]\) as described in \([37]\).
For Defuzzification, a conversion of fuzzy inferences or output node expression value predictions, in fuzzy space, back to regular or crisp values, we employed the simplified centroid method [27]. Given a predicted fuzzy values of an output node \( y = [y_1, y_2, y_3] \), we defined defuzzified expression values (\( \bar{x} \)) as:

\[
\bar{x} = \frac{y_3 - y_1}{y_1 + y_2 + y_3}
\]

After defuzzification, we reverse transformed back to \( \log_2 \) expression values by multiplying derived values by \( \frac{\pi}{2} \) and applying the tangent function.

### 2.2.1 Inferred regulatory model fit or error

\( \text{jFuzzyMachine} \) estimates the an error of the fit for \( M \) samples or perturbations of an output feature \( x = \{x_1, x_2, ..., x_M\} \) as:

\[
E = 1 - \frac{\sum_{i=1}^{M} (x_i - \tilde{x}_i)^2}{\sum_{i=1}^{M} (x_i - \bar{x})^2}
\]

where \( \tilde{x} = \{\tilde{x}_1, \tilde{x}_2, \cdots, \tilde{x}_M\} \) is the set of defuzzified numerical log expression ratios predicted for the output feature and \( \bar{x} \) is the mean of the experimental values of \( x \) across the samples or perturbations observed. A perfect fit would result in a maximum \( E \) of 1.0. \( \text{jFuzzyMachine} \) uses the estimated error to rank possible models of interactions that predict on output node.

### 2.3 Design and Implementation

The \( \text{jFuzzyMachine} \) tool is implemented in the platform-independent Java programming language to facilitate an extensive community reach. It is modular in design to facilitate an easy decoupling of component parts. It consists of:
1) the Initiation Module, 2) the Main Module, and 3) the Utilities Module (see Figure 1). The ‘Initiation Module’ consists essentially of the program configuration and run parameter specification units. Depending on user-desired added-functionality beyond regulatory model inference, a user may choose to specify parameters that apply only to desired post-inference processing. The Main Module houses the application’s main functionality – the fuzzy logic based regulatory inference engine. The module implements the: fuzzification, rule evaluation, and defuzzification schemes [31, 46][29]. It currently implements the ‘Union Rule Configuration’ (URC) rule evaluation scheme of Coomb’s et al [8, 36] and an optimized version of the ‘Exhaustive search’ algorithm of Sokhansaj et al [?]. The Utilities Module consists of two submodules: a) the Postprocessing Submodule and, b) the Add-ons (or Plug-ins) Submodule. The Postprocessing Submodule consists of three Units – the ‘Graph’, ‘Evaluation (or Validation)’, and the ‘Dynamic Simulation’ Units. The Graph Unit consolidates the best fitted models derived from the fuzzy inference system into a network graph as explained in Gormley et al [15]. The Evaluation Unit simply compares expression profile predictions by inferred models to the experiment observed values. Depending on the user-specification, this may be against original model elucidation data (default) or an independent dataset. Also depending on the user, a re-calculation of the model’s fit may be specified, particularly to quantitatively describe how well models fit independent datasets. The ‘Dynamic Simulation Unit’ implements and executes model dynamic simulations as also described in Gormley et al [15]. To facilitate downstream data integration, the dynamic simulations’ stop criteria is dependent on the user-specified number of iteration steps and not the computed error. However the error estimates at the end of the simulation runs are reported. In anticipation of community contributions, the Add-on (or Plug-ins) Submodule is described. Current in-house created
Figure 1: The jFuzzyMachine Application Components

functionalities that would fit appropriately configured add-on units include an “In-Silico Knockout Simulations” add-on and a “Visualization” add-on which depends on a secondary-installed program. These are also freely available on request.

2.4 Getting jFuzzyMachine

jFuzzyMachine’s source codes and precompiled binaries are freely available at the Github repository locations https://github.com/paiyetan/jfuzzymachine
and https://github.com/paiyetan/jfuzzymachine/releases/tag/v1.7.21 respectively.

2.5 Installation Requirements

jFuzzyMachine is platform independent. It would run on a Windows, Mac, or UNIX-based Operating System (OS) with an appropriately preinstalled Java Runtime Environment (JRE). Java 7 or above is required. You may download the latest version of Java from https://www.java.com/en/download/.

To run the visualization add-on (plugin), provided as an added-value, a UNIX-based OS with the R program statistical computing environment preinstalled, is required. R may be downloaded from https://cran.r-project.org/.

2.6 Installing jFuzzyMachine

Unzip the compressed application package into a directory of choice. The content of the unzipped folder should include: One primary java archive (.jar) folder, four runtime configuration (.config) files, and four subdirectories (etc/, lib/, plugins/, and src/),

- JFuzzyMachine.jar
- jfuzzymachine.config
- jfuzzymachine.graph.config
- jfuzzymachine.evaluator.config
- jfuzzymachine.simulator.config
- etc
- lib
- plugins
- src
The configuration files are pre-filled to satisfy required parameters for this manual’s demonstration. Users may appropriately fill-in their own specifications and experiment with the tool. See configuration options below.

2.7 Running jFuzzyMachine

To run the tool, on the command-line,

1. Navigate into the application directory

2. Appropriately fill-in the desired run-time options in the configuration files and

3. Depending on application module or functional unit of interest, type the following commands, one at a time:

To elucidate fuzzy logic-based regulatory relationships, run the commands

```java
java -Xmx10G -cp JFuzzyMachine.jar jfuzzymachine.
JFuzzyMachine jfuzzymachine.config
```

To derive a composite network graph, including rule frequencies, run

```java
java -Xmx10G -cp JFuzzyMachine.jar jfuzzymachine.
utilities.graph.Graph jfuzzymachine.graph.config
```

To evaluate or validate how well inferred fuzzy logic-based regulatory models fit the data, run

```java
java -Xmx10G -cp JFuzzyMachine.jar jfuzzymachine.
utilities.ModelValidator jfuzzymachine.evaluator.config
```
To run dynamic simulation of regulatory network, and tease expression values at systems steady state, run

```
java -Xmx10G -cp JFuzzyMachine.jar jfuzzymachine.
utilities.simulation.Simulator \
   jfuzzymachine.simulator.config
```

The -Xmx10G option indicates the amount of memory space available to the program during the program execution. It is specified as 10 Gigabytes. For very large expression data, it is recommended to be increased. The -cp option indicates the location of our Java program class files – specified as JFuzzyMachine.jar. It is assumed this is located in the current working directory.

### 2.7.1 The jfuzzymachine.config file

```
## jfuzzymachine.config
inputFile=./etc/projects/demo/inputs/exprsMat.txt
outputDir=./etc/projects/demo/outputs/runJFuzzy
maxNumberOfInputs=-1
numberOfInputs=3
outputInRealtime=TRUE
eCutOff=0.6
useAllGenesAsOutput=FALSE
iGeneStart=1
iGeneEnd=14
useParallel=TRUE
```

The jfuzzymachine.config has, at least, the above listed parameters (‘key’=‘value’ pairs). The associated values listed here are for demonstration purposes in this manual. The inputFile option specifies the relative path to the data matrix of
normalized expression values. The outputDir specifies the path to the directory where results from jFuzzyMachine are to be placed. The maxNumberOfInputs option is a flag that specifies how jFuzzyMachine should handle input (regulatory) features. A negative flag indicates that exactly the specified numberOfInputs option be considered. A positive value specifies to jFuzzyMachine to consider all possible number of inputs up-to the specified value. E.g. a positive value of 4, simply says to jFuzzyMachine to consider all possible combinations of 1, 2, 3, and 4 regulatory inputs to an output feature. Current implementation of jFuzzyMachine allows up to 5 inputs. A negative flag however, says to jFuzzyMachine to consider only possible combinations of 3 regulatory inputs (specified by the numberOfInputs option in the above configuration) to an output feature. The outputInRealtime option tells jFuzzyMachine to output its runtime informations onto a standard output (the console). This typically includes derived models, inferred regulatory rules, and computed fit estimates. The eCutOff option specifies the cut-off for which to consider a computed fuzzy logic model. Models below the specified value are discarded. The useAllGenesAsOutputs option specifies whether to consider all features in the expression values matrix or a limited set specified by the iGeneStart and iGeneEnd options. The iGeneStart and iGeneEnd options specify the range of features to use from a numerically ordered list – the expression matrix row numbers. The options iGeneStart=1 and iGeneEnd=14 in the configuration above, simply says to jFuzzyMachine to consider features of expression profiles from the first to the 14th row, in the expression matrix (inputFile), as probable outputs in the regulatory model inference. The useParallel option indicates whether to run jFuzzyMachine in the optimized mode for speed (distributed across computing cores available at runtime).

2.7.2 The jfuzzymachine.graph.config file

```bash
## jfuzzymachine.graph.config
```
The above are parameters (runtime options) to the jFuzzyMachine Graphical unit. The exprsFile option specifies a path to the expression matrix from which regulatory models were inferred. The input option specifies a path to the directory in which jFuzzyMachine inferred models and output result files are placed. The runId option is a user-specified identifier prepended to outputted results’ filenames (please see the Results section). The nomenclature (naming convention) of the outputted result files is of the form <runId>_runJFuzzUtils.<fileType>. The fitCutOff option specifies a cut-off for considering models. Models above specified fitCutOff are considered for inclusion in a consolidated network model. Only the regulatory edges of the passing models are considered. The useAnnotatedGraphModel tells jFuzzyMachine Graph unit to model regulatory network as a directed acyclic graph. If TRUE, the outputted adjacency matrix (.adj or .mat file) is a directed graph. By default, jFuzzyMachine’s Graphical unit outputs only an adjacency matrix file, to represent the inferred regulatory network, but the option outputEdges specifies to jFuzzyMachine to also print edges (a .edg file) of the consolidated network. The topFittedModelsToOutput applies to outputting fitted models. It specifies the number of alternate top ranked models that pass fitCutOff filter option to report in the output (.fit2) file.

2.7.3 The jfuzzymachine.evaluator.config file

```bash
## jfuzzymachine.evaluator.config
exprsToValidate=./etc/projects/demo/inputs/exprsMat.txt
```
In jfuzzymachine.evaluator.config file, the exprsToValidate option specifies a path to the expression matrix from which regulatory models were inferred – in the case of evaluating the performance of the inferred models against the model-generating data. For an independent evaluation of the model, this is a path to the expression matrix of the independent dataset. The fitFile specifies the path to derived .fit file from jFuzzyMachine’s Graph unit. The .fit file contains the best fitted models. The fitCutOff specifies an estimated fit cut-off value above which to consider models. The validationType option, specifies what validation is being performed. Acceptable values include validations (default) and invalidations. Specifying invalidations implies an independent validation is being performed.

2.7.4 The jfuzzymachine.simulator.config file

In the jfuzzymachine.simulator.config, the exprsMatFile specifies a path to the expression matrix file.
expression matrix from which regulatory models were inferred – in the case of evaluating the performance of the inferred models against the model-generating data. For an independent evaluation of the model, this is a path to the expression matrix of the independent dataset. The `edgesFile` and `fitFile` options specify the path to the `.edg` and `.fit` files derived from `jFuzzyMachine`'s Graph unit. These contain the edges of the consolidated network and the best fitted models from the regulatory model elucidating steps. The `fitCutOff` specifies an estimated fit cut-off value above which to consider models. The `simulationType` option specifies the sort of dynamic simulation to be performed. Acceptable values include `simulations` (default) and `isimulations`. Specifying `isimulations` implies a dynamic simulation of consolidated network, using previously derived models as simulation parameters on an independent dataset is being performed. The `maxIterations`, `eCutOff`, `initialOutputsValues` and `alpha` are other dynamic simulation parameters. The `maxIteration` and `eCutOff` are the stopping criteria – maximum iteration steps and error estimate cut-off respectively. Default values are 5000 and $10^{-7}$ respectively. To better facilitate integration with downstream analyses, the Dynamic Simulation Unit defaults to preferably using the `maxIteration` option as stopping criteria. The `initialOutputsValues` specifies which 'perturbation', 'sample', or 'time-point' values, from the expression matrix, to use as initial values in the simulation. It defaults to `ALL`, i.e. all values are sequentially used. Other values are `FIRST` and `RANDOM`, implying the first column and a random column values respectively. The `alpha` option specifies the 'mixing parameter', $\alpha$, of the simulation model. Based on Gormley et al, linear combination of new and old values ensures that the system smoothly converges towards equilibrium. And accordingly, `jFuzzyMachine`'s Dynamic Simulation Unit computes new values of each node ($I_1$) based on the initial conditions and the fuzzy relations inferred from the data; values in the next iteration were calculated as...
a linear combination of the inferred values ($I_n$) and the initial values ($I_{n-1}$) as follows:

$$I_{n+1} = \alpha I_n + (1 - \alpha) I_{n-1}$$

(1)

### 2.8 Comparing jFuzzyMachine’s inferred network to ARACNe’s

To compare regulatory network inferred by jFuzzyMachine with that inferred by the ARACNe (an Algorithm for the Reconstruction of Gene Regulatory Networks algorithm [26], mutual information matrix of sampled features expression profile was inferred using the R/bioconductor minet package build.mim routine and specifying the spearman option as the estimator. The minet package ARACNe algorithm implementation was used to derived weighted adjacency matrix of the inferred network. The identified edges were compared to those inferred from the best fitted models from a jFuzzyMachine inference, given the same expression profile.

### 3 RESULTS AND DISCUSSION

In this section we describe results outputted from a typical jFuzzyMachine execution. These are are described as results produced by the different modules of the tool - the Main module and Utilities module. Presented results are those of the sampled data expression profile.

In the second part, we describe, the results obtained from a comparison of the results of jFuzzyMachine and that of the ARACNe algorithm with respect to potential regulatory interactions derived from the sampled data.
3.1 The Main Module - Inference Engine

The main output results from the jFuzzyMachine inference engine are written to the outputDir. These are files or single file ending with .jfuz. From the demo run, this would be the ./etc/projects/demo/outputs/runJFuzzy/exprsMat.1.14.3.TRUE.jfuz. This consists of 4 major sections indicated by the > character at the beginning of the line. These sections include: a prologue, run parameter listing, the main result, and an epilogue. The prologue section stores information such as the run’s start-time, while the epilogue stores the run end-time and duration. The main section is a tab-delimited table with columns: Output, NumberOfInput(s), Input(s), Rule(s), and Error(E). The Output column indicates the output node in the model; the NumberOfInput indicate the number of input nodes, the Input(s), considered. The Rule(s) column indicate the fuzzy logic rule that associates the respective input node to the output node. The Error(E) column indicates the model’s fit. Shown below is a sample output from the demo run:

```bash
> StartTime: Mon Jul 20 23:47:25 EDT 2020
> Search Parameters:
  inputFile = ./etc/projects/demo/inputs/exprsMat.txt
  maxNumberOfInputs = -1
  numberOfInputs = 3
  outputInRealtime = TRUE
  eCutOff = 0.6
  useAllGenesAsOutput = FALSE
  iGeneStart = 1
  iGeneEnd = 14
  useParallel = TRUE
  outputFile = ./etc/projects/demo/outputs/runJFuzzy/exprsMat.1.14.3.TRUE.jfuz
  modelPhenotype = FALSE
```
Initiating...
Searching (Exhaustive Search)...
  All Genes#: 14
  Output Nodes Considered#: 14
> Begin Search Result Table:
Output NumberOfInput(s) Input(s) Rule(s) Error (E)
PTHLH 3 [KRT86, RUBCNL, CYS1] [[1, 3, 3], [1, 3, 2], [2, 1, 1]] 0.6057812852061071
PTHLH 3 [LINC00707, RUBCNL, LINC00634] [[1, 1, 1], [1, 2, 2], [2, 1, 1]] 0.6395610849445412
PTHLH 3 [LINC00707, RUBCNL, LINC00634] [[1, 1, 1], [1, 2, 3], [1, 1, 1]] 0.60556246580613
PTHLH 3 [LINC00707, RUBCNL, LINC00634] [[1, 1, 1], [1, 3, 2], [2, 1, 1]] 0.7444440833291
PTHLH 3 [LINC00707, RUBCNL, LINC00634] [[1, 1, 1], [3, 2, 3], [2, 1, 1]] 0.6035443648873104
PTHLH 3 [LINC00707, RUBCNL, LINC00634] [[2, 1, 1], [1, 2, 2], [2, 1, 1]] 0.664214821186895
PTHLH 3 [LINC00707, RUBCNL, LINC00634] [[2, 1, 1], [1, 3, 2], [1, 1, 1]] 0.6474161595899811
PTHLH 3 [LINC00707, RUBCNL, LINC00634] [[2, 1, 1], [1, 3, 2], [2, 1, 1]] 0.7321145800582836
...
C2orf78 3 [SERPINB7, CYS1, LINC00886] [[3, 3, 1], [1, 2, 3], [1, 3, 3]] 0.6316025202379136
LINC00634 3 [LINC00886, GCNT4, MGAM] [[1, 3, 3], [3, 2, 1], [2, 1, 1]] 0.6000086433874239
LINC00634 3 [KRT86, LINC00886, GCNT4] [[3, 1, 1], [2, 2, 3], [3, 1, 1]] 0.6009588086189968
> End Search Result Table

...Done!
> Epilogue

Started: 1595303245550: Mon Jul 20 23:47:25 EDT 2020
3.2 The Utilities Module

jFuzzyMachine’s Utilities Module consists of the 'Postprocessing' and the 'Add-ons' submodules. The postprocessing module consists of the 'Graph', 'Evaluation' and 'Dynamic Simulations' Units.

3.2.1 The Graph Unit

Outputs from the graph unit execution are placed in the runJFuzzUtils subdirectory. With regards to this demonstration, this would be the .*/etc/projects/demo/outputs/runJFuzzy/runJFuzzUtils directory. These tab-delimited result files include:

- _demo_runJFuzzUtils.adj
- _demo_runJFuzzUtils.edg
- _demo_runJFuzzUtils.edg2
- _demo_runJFuzzUtils.fit
- _demo_runJFuzzUtils.fit2
- _demo_runJFuzzUtils.fre

The _demo_runJFuzzUtils.adj file is a directed graph adjacency matrix describing the connections in the inferred network. A connection between two nodes is indicated by 1 and 0 vice versa. Features in the rows are the inputs while those in columns are the output nodes. The _demo_runJFuzzUtils.edg and _demo_runJFuzzUtils.edg2 result files are about the same. Describing the edges in the inferred network, they both have the columns; From, To, Rule, and Weight in common. These correspond to the input node, output node, fuzzy logic rule associating the input with the output node, and estimated model fit respectively.
The ‘HashCode’ column in the "edg" file is only included for programmatic debugging. Likewise, the `demo_runJFuzzUtils.fit` and `demo_runJFuzzUtils.fit2` result files are about the same. While the .fit reports all models above the specified fitCutOff in the `jfuzzymachine.graph.config` file, the .fit file reports only the best fitted model to each output node. Sampled outputs from the related demo run are shown below:

```
# _demo_runJFuzzUtils.fit
Output  NumberOfFittedModels  InputNodes(BestFit)  Rules  Fit
C2orf78 7 [SERPINB7, CYS1, MGAM] [[3, 3, 1], [1, 2, 3], [3, 1, 1]] 0.7246192458666514
LINCO0634 2 [KRT86, LINCO0886, GCNT4] [[3, 1, 1], [2, 2, 3], [3, 1, 1]] 0.6009588086189968
...
```

```
# _demo_runJFuzzUtils.fit2
Output  InputNodes  Rules  Fits
C2orf78 [SERPINB7, CYS1, MGAM] [[3, 3, 1], [1, 2, 3], [3, 1, 1]] 0.7246192458666514
C2orf78 [SERPINB7, CYS1, MGAM] [[3, 2, 1], [1, 2, 3], [3, 1, 1]] 0.646429505593284
...
SERPINB7 [ROBO4, C2orf78, GCNT4] [[3, 2, 3], [3, 2, 1], [1, 3, 3]] 0.6002583965182628
SERPINB7 [ROBO4, C2orf78, LINCO0634] [[1, 2, 3], [3, 1, 1], [2, 3, 1]] 0.6002285026737246
SERPINB7 [PTHLH, CYS1, C2orf78] [[1, 3, 1], [3, 1, 1], [3, 2, 1]] 0.600143816707301
PTHLH [LINCO0707, RUBCNL, LINCO0634] [[1, 1, 1], [1, 3, 2], [2, 1, 1]] 0.7444440833291
PTHLH [LINCO0707, RUBCNL, LINCO0634] [[2, 1, 1], [1, 3, 2], [2, 1, 1]] 0.7321145800582836
...
```

The `demo_runJFuzzUtils.fre` reports the frequency of the fuzzy rules eval-
uated in the inferred models with an estimated fit value above the fitCutOff. Please see Gormley et al and Sokhansanj et al for a detailed explanation of the rules.

3.2.2 The Evaluation Unit

The Evaluation Unit compares expression profile predictions by inferred models to an experiment values – either the fuzzy logic models’ model-elucidating data or an independent dataset. Its output are reported in the _demo_runJFuzzUtils.val file – an expression matrix of predicted values of output nodes, given the values in the exprsToValidate file and the set of fuzzy logic models in the fitFile specified in the jfuzzymachine.evaluator.config.

3.2.3 The Dynamic Simulation Unit

The ‘Dynamic Simulation Unit’ implements and executes model dynamic simulations as also described in Gormley et al. The unit implements an iterative scheme to determine the state of the network at equilibrium. Simulation values are reported in the runJFuzzyUtils/simulations/subsub-directory in the jFuzzy-Machine main output directory. These are captured in the .dta and .sim files. The .dta files report the error values following each iteration, while the .sim file reports the estimate for each output node in the network at each iteration. The numerical value in the naming convention of the derived files show the column index, in the expression matrix, of the ‘sample’, ‘perturbation’, or ‘time-point’ from which initial values for the respective simulations were derived.

3.3 Add-ons

jFuzzyMachine and its outputs are designed to either be standalone resources, or be easy to integrate with other analyses pipelines and platforms. Add-ons or
plug-ins provide an avenue to easily integrate additional functionalities or integrate other tools to the base application. For a better appreciation of results, we have included an example plug-in to enable some visualization of results demonstrated in this manual. As previously stated (please see publication), plug-ins can be platform dependent and may rely on secondary applications for full functionality. The plug-in bundled with jFuzzyMachine requires a UNIX-based platform or OS with the R statistical programming environment pre-installed. In addition to having the R program pre-installed, the following R/Bioconductor packages are required:

- optparse
- org.Hs.eg.db
- xtable
- igraph
- graph
- Rgraphviz
- pheatmap
- ReactomePA

To execute, simply run the following commands from within the jFuzzyMachine application working directory:

```
1 plugins/viz/rJFuzzyMachineUtilsExec.sh
2 plugins/viz/rJFuzzyMachineUtilsNetworkExec.sh
```

Example output figures, saved in the ./etc/projects/demo/outputs/plugins/viz/figs directory are presented below:

Figure 2 shows the predicted values by the best fitted models for a the sample output node, the C2orf78 gene product. It can be appreciated that predicted
values across the assay samples tend to trend similarly as the observed values indicated in gray. jFuzzyMachine predictions are model-based. A model consists of a set on input nodes and an output node with regulatory rule relationships among these.

Figure 3 shows predictions at steady states of an inferred regulatory network. Observed convergence gives a bit of added assurance that inferred relationships are likely to be feasible. A lack of convergence may indicate a suboptimal network inference secondary to suboptimal fits of derived models.

Figures 4 and 5 show the inferred networks of the jFuzzyMachine and that of the ARACNe algorithm respectively. It is observed that jFuzzyMachine both appear to have a large overlap in the number of predicted edge relationships. Fig. 6. jFuzzyMachine almost always predict the direction of relationship (i.e. what is regulating what). Given a evidences from the two tools of potential interaction between pairs of features, the biological significance of such interactions may be worth further pursuit.

4 CONCLUSION AND RECOMMENDATION

The Fuzzy logic inference approach to elucidating regulatory networks, although relatively mature, has little to no freely available tool to facilitate its adoption, to a greater extent, in elucidating high-throughput biological expression data. The jFuzzyMachine tool fills this apparent need. jFuzzyMachine mitigates the limitation of applying and appropriating the benefits of the fuzzy inference system to biological data. With respect to predicting regulatory interactions, jFuzzyMachine compares favorably with other regulatory inference tools. We expect to continue to include added functionalities and improve its current implementation. With our modular design and plan to accommodate third-party
Figure 2: An Evaluation (Validation) Plot. A visual evaluation of predictions of a fitted model for a sample output node, the **C2orf78** gene. The estimated fit was 0.72. The input (regulatory) nodes were the genes **SERPINB7**, **CYS1**, and **MGAM**. The y-axis indicates the normalized expression values and the a-axis indicates the sample perturbations or treatment. Samples were reverse transfected vorinostat-resistant colon cancer, HCT-116, cell lines. Each sample was treated with the indicated small interfering ribonucleic acid (siRNA) to knockdown the respectively indicated gene products. The grey plot line shows the observed expression profile of the gene **C2orf78**, while the "red" line shows the predicted expression value from the expression of the regulators in the given data, and the rules associating the regulators to the output. The inferred patterns of regulation (rules) are indicated in Figure 4. It can apparently be appreciated that the fuzzy logic model is able to tease out trend in the dataset.
Figure 3: A Dynamic Simulation Plot. Having randomly chosen a sample (the 25th column sample) from the normalized expression matrix to provide initial values of expression, and given the best fitted models, the plot shows predicted expression values for the inferred outputs KRT86, PTHLH, SERPINB7, C2orf78, CYS1 and LINC00634 over 5000 iterations. It is appreciable that the inferred network achieves an equilibrium state at a little over 1000 iterations, when a change in predicted values tend to zero.
Figure 4: The Fuzzy Logic-based Regulatory Network Inferred. A composite regulatory network is inferred from the best fitted models for each node. The inferred network consists of 13 nodes (genes), and 18 edges (regulatory connections). The arrow heads indicate the regulatory direction from the input node to the output node. The edge labels, shown by the fuzzy rules, indicate the regulatory interaction. From Gormley et al, Rule configuration is the specification of if-then relationships between variables in fuzzy space. For example, an inhibitory relationship is represented by the rule vector $r = [r_1, r_2, r_3] = [3, 2, 1]$ (i.e., if input is low ($r_1$), then output is high (3); if input is medium ($r_2$), then output is medium (2), and if input is high ($r_3$), then output is low (1). From the composite regulatory network, the regulatory effect of the MGAM gene on the C2orf78 gene is indicated by the rule 3, 1, 1. This implies that when MGAM is low ($r_1$), C2orf78 is high (3); when it is medium ($r_2$), C2orf78 is low (1); and when MGAM is high ($r_3$), C2orf78 is low (1). Notice that the bi-directional relationship between the pair of genes C2orf78–CYS1, and C2orf78–SERPINB7.
Figure 5: The ARACNe-inferred Regulatory Network. As described in the Materials and Methods section, mutual information between pairs of features was estimated using the correlation approach, to derive a mutual information matrix.
Figure 6: Identified edges between jFuzzyMachine and the ARACNe algorithm. A total of 16 edge relationships were identified by jFuzzyMachine. Two of these are observed to be by directional (Fig 2). The ARACNe algorithm identifies 14 network edges, over 70 percent of which are equally identified by jFuzzyMachine.
add-ons, we hope to facilitate community contributions and a scientific ecosystem of adopters. Also, we hope to continue to facilitate its integration with other high-throughput biological inference tools.

5 ACKNOWLEDGEMENT

A special thanks to Dr. Iosif Vaisman, Dr. Dmitri Klimov, Dr. Saleet Jafri, and Dr. Quong at the George Mason University School of Systems Biology, for mentoring a larger body of the work for which a component part is presented here. The content presented here is solely the responsibility of the author and does not reflect the official views of any affiliated institutions.

6 AVAILABILITY

The jFuzzyMachine source codes and binaries are freely available at the Github repository locations: https://github.com/paiyetan/jfuzzymachine and https://github.com/paiyetan/jfuzzymachine/releases/tag/v1.7.21.

References

[1] Tuqyah Abdullah Al Qazlan, Aboubekeur Hamdi-Cherif, and Chafia Karam Mohamed. State of the art of fuzzy methods for gene regulatory networks inference. Scientific World Journal, 2015:148010, 2015.

[2] Simon Andrews. {FastQC}: A quality control tool for high throughput sequence data. \url{https://www.bioinformatics.babraham.ac.uk/projects/fastqc/}.

[3] Peter Atkins, Julio Paula, and James Keeler. Atkin’s Physical Chemistry. Oxford University Press, Oxford, 2018.
[4] Guy N Brock, Vasyl Pihur, and Laura Kubatko. Detecting Gene Regulatory Networks from Microarray Data Using Fuzzy Logic, 2009.

[5] Kuang-Chi Chen, Tse-Yi Wang, Huei-Hun Tseng, Chi-Ying F Huang, and Cheng-Yan Kao. A stochastic differential equation model for quantifying transcriptional regulatory network in Saccharomyces cerevisiae. Bioinformatics, 21(12):2883–2890, jun 2005.

[6] Ting Chen, Hongyu L He, and George M Church. {MODELING} {GENE} {EXPRESSION} {WITH} {DIFFERENTIAL} {EQUATIONS}, 1998.

[7] Ye Chen, Lawrence J Mazlack, and Long J Lu. Inferring Fuzzy Cognitive Map models for Gene Regulatory Networks from gene expression data, 2012.

[8] W E Combs and J E Andrews. Combinatorial rule explosion eliminated by a fuzzy rule configuration, 1998.

[9] Suman Datta and Bahrad A Sokhansanj. Accelerated search for biomolecular network models to interpret high-throughput experimental data. BMC Bioinformatics, 8:258, jul 2007.

[10] Wélliton de Souza, Wélliton de Souza, Benilton de Sá Carvalho, and Iscia Lopes-Cendes. Rqc: A Bioconductor Package for Quality Control of {High-Throughput} Sequencing Data, 2018.

[11] P D’haeseleer, X Wen, S Fuhrman, and R Somogyi. Linear modeling of {mRNA} expression levels during {CNS} development and injury. Pac. Symp. Biocomput., pages 41–52, 1999.

[12] P Du, J Gong, E SyrkinWurtele, and J A Dickerson. Modeling Gene Expression Networks Using Fuzzy Logic, 2005.
[13] Vladmir Filkov, Steven Skiena, and Jizu Zhi. Analysis techniques for microarray time-series data, 2001.

[14] J P Fitch and B Sokhansanj. Genomic engineering: moving beyond {DNA} sequence to function. Proc. IEEE, 88(12):1949–1971, 2000.

[15] Michael Gormley, Viswanadha U Akella, Judy N Quong, and Andrew A Quong. An integrated framework to model cellular phenotype as a component of biochemical networks. Adv. Bioinformatics, 2011:608295, nov 2011.

[16] Raed I Hamed. Computational Modeling and Dynamical Analysis of Genetic Networks with {FRBPN-} Algorithm, 2011.

[17] Alboukadel Kassambara. fastqcr: Quality Control of Sequencing Data. {R} package version 0.1.2, 2019.

[18] Daehwan Kim, Geo Pertea, Cole Trapnell, Harold Pimentel, Ryan Kelley, and Steven L Salzberg. {TopHat2}: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol., 14(4):R36, apr 2013.

[19] Y Kodama, M Shumway, R Leinonen, and on behalf of the International Nucleotide Sequence Database Collaboration. The sequence read archive: explosive growth of sequencing data, 2012.

[20] R Leinonen, H Sugawara, M Shumway, and on behalf of the International Nucleotide Sequence Database Collaboration. The Sequence Read Archive, 2011.

[21] J-C Leloup and A Goldbeter. Toward a detailed computational model for the mammalian circadian clock, 2003.
[22] Yang Liao, Gordon K Smyth, and Wei Shi. The \{R\} package Rsubread is easier, faster, cheaper and better for alignment and quantification of \{RNA\} sequencing reads. *Nucleic Acids Research*, 47(8):e47, may 2019.

[23] Michael I Love, Wolfgang Huber, and Simon Anders. Moderated estimation of fold change and dispersion for \{RNA-seq\} data with \{DESeq2\}. *Genome Biol.*, 15(12):550, 2014.

[24] P C H Ma and K C C Chan. Inferring Gene Regulatory Networks From Expression Data by Discovering Fuzzy Dependency Relationships, 2008.

[25] Patrick C H Ma and Keith C C Chan. A Fuzzy Data Mining Technique for the Reconstruction of Gene Regulatory Networks from Time Series Expression Data, 2006.

[26] Adam A Margolin, Ilya Nemenman, Katia Basso, Chris Wiggins, Gustavo Stolovitzky, Riccardo Dalla Favera, and Andrea Califano. ARACNE: An Algorithm for the Reconstruction of Gene Regulatory Networks in a Mammalian Cellular Context, 2006.

[27] J M Mendel. Fuzzy logic systems for engineering: a tutorial, 1995.

[28] R Core Team. *R: A language and environment for statistical computing*, 2019.

[29] Khalid Raza. Fuzzy logic based approaches for gene regulatory network inference. *Artif. Intell. Med.*, 97:189–203, jun 2019.

[30] Mark Reimers and Vincent J Carey. [8] Bioconductor: An Open Source Framework for Bioinformatics and Computational Biology, 2006.

[31] H Ressom, D Wang, R S Varghese, and R Reynolds. Fuzzy logic-based gene regulatory network, may 2003.
[32] Apadmmuhammad Shoaib B Sehgal, Iqbal Gondal, and Laurence S Dooley. {CF-GeNe}: Fuzzy Framework for Robust Gene Regulatory Network Inference, 2006.

[33] M S B Sehgal, I Gondal, L Dooley, and R Coppel. {AFEGRN}: Adaptive Fuzzy Evolutionary Gene Regulatory Network Re-construction Framework, 2006.

[34] Muhammad Shoaib B Sehgal, Iqbal Gondal, Laurence Dooley, and Ross Coppel. Coalesce Gene Regulatory Network Reconstruction: A {Cross-Platform} Transcriptional Gene Network Fusion Framework, 2006.

[35] Lev A Soinov, Maria A Krestyaninova, and Alvis Brazma. Towards reconstruction of gene networks from expression data by supervised learning. *Genome Biology*, 4(1):R6, 2003.

[36] B A Sokhansanj and J P Fitch. {URC} fuzzy modeling and simulation of gene regulation.

[37] Bahrad A Sokhansanj, J Patrick Fitch, Judy N Quong, and Andrew A Quong. Linear fuzzy gene network models obtained from microarray data by exhaustive search. *BMC Bioinformatics*, 5:108, aug 2004.

[38] Bahrad A Sokhansanj, Janine B Garnham, and J Patrick Fitch. Interpreting microarray data to build models of microbial genetic regulation networks, 2002.

[39] The SAM/BAM Format Specification Working Group. Sequence {Alignment/Map} Format Specification.

[40] Cole Trapnell, Lior Pachter, and Steven L Salzberg. {TopHat}: discovering splice junctions with {RNA-Seq}. *Bioinformatics*, 25(9):1105–1111, may 2009.
[41] Cole Trapnell, Brian A Williams, Geo Pertea, Ali Mortazavi, Gordon Kwan, Marijke J van Baren, Steven L Salzberg, Barbara J Wold, and Lior Pachter. Transcript assembly and quantification by {RNA-Seq} reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol.*, 28(5):511–515, may 2010.

[42] John J Tyson. Models of cell cycle control in eukaryotes, 1999.

[43] L G Volkert and N Malhis. An efficient method for fuzzy identification of regulatory events in gene expression time series data.

[44] Fei Wang, De Pan, and Jianhua Ding. A New Approach Combined Fuzzy Clustering and Bayesian Networks for Modeling Gene Regulatory Networks, 2008.

[45] D C Weaver, C T Workman, and G D Stormo. Modeling regulatory networks with weight matrices. *Pac. Symp. Biocomput.*, pages 112–123, 1999.

[46] P J Woolf and Y Wang. A fuzzy logic approach to analyzing gene expression data. *Physiol. Genomics*, 3(1):9–15, jun 2000.

[47] Ronald R Yager and Lotfi A Zadeh. *An Introduction to Fuzzy Logic Applications in Intelligent Systems*. Springer Science & Business Media, dec 2012.

[48] Lotfi A Zadeh. Fuzzy sets. *Information and Control*, 8(3):338–353, 1965.

[49] Lotfi Asker Zadeh and Rafik Aziz Ogly Aliev. *Fuzzy Logic Theory and Applications: Part {I} and Part {II}*. 2018.

[50] Lotfi Asker Zadeh, George J Klir, and Bo Yuan. *Fuzzy Sets, Fuzzy Logic, and Fuzzy Systems: Selected Papers*. World Scientific, 1996.