Plugin Based Microbiome Analysis (PLuMA, formerly MiAMi)
Version 1.0 - User Guide

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Abstract

We present PLUMA, a lightweight and flexible package for constructing general software pipelines. PLUMA is designed to be infinitely extensible, allowing researchers to select a specific set of dynamically loaded plugins as sequential stages of their pipelines. These plugins can be implemented by either themselves or other users, in their language of choice.

We begin by introducing the key features of PLUMA, and follow with a discussion of how to download and install the latest version, compile, and run the software. We also include information on setting up configuration files that specify desired plugins for a pipeline, and how to extend PLUMA with new plugins in various programming languages. Finally, we conclude with a full pipeline example and a brief discussion of our envisioned future of PLUMA.

We distribute PLUMA under the GNU GENERAL PUBLIC LICENSE (GPL-3), copyrighted by Florida International University.
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Chapter 1

Introduction

Software pipelines are applicable to any field of study and involve a framework where control flow executes as a series of stages, with an output of a given stage serving as an input to the next. As an example, Figure 1.1 shows a common pipeline in metagenomics analysis, with an initial set of DNA sequences passing through a denoising stage that improves the quality of reads, followed by clustering through some similarity or compositional metric, and finally labelling sequence clusters with the closest matching taxonomic unit [2]. Each stage takes as input the output of the previous stage, and produces new output.

Many pipelines have been developed by independent teams as standalone tools, although stages of individual pipelines could potentially be reused amongst one another. Particularly in the field of metagenomics analysis, there are many software pipelines that perform similar tasks and may even share stages that are still constructed independently, because there is no standardized framework for developing, testing and particularly integrating these stages. PLUMA is designed to address this need by providing a lightweight and generic back end that can execute these stages as dynamically loaded plugins, specified by a user through a configuration file or GUI.

We show the conceptual design of PLUMA in Figure 1.2. The design of PLUMA follows that of a Problem-Solving Environment (PSE, [21]) using three tiers: a compiled machine layer, a middle scripted layer, and an upper user layer. PSEs are specifically designed with extensibility in mind, but often involve only two layers. The first is what we refer to as the machine layer (also often called a back end), and the other is what we refer to as the user layer (also often called a front end). The user layer of PLUMA consists of an easy-to-use configuration file, and we plan on adding a graphical interface later. The machine layer consists of compiled plugin extensions to the software that become integrated with the back end through application of various software design patterns [22].

PSEs are hierarchical, so that any upper layer can invoke any of the layers beneath. What distinguishes PLUMA from two-tier packages is the middle (scripting) layer between the user and computational layers. This improves extensibility by allowing new plugins to be built using components of existing plugins, which

![Diagram](image)

Figure 1.1: An example metagenomics analysis pipeline. Each stage gets executed sequentially, with the output of a specific stage serving as input to a later stage of the pipeline.

5
facilitates development of new plugins while minimizing having to reinvent the wheel. It has been shown through case studies [14] that this three-tier design offers improved extensibility for both biologists and computer scientists.

Moreover, this design allows users to develop new plugins in the language of their choice. Currently, PLUMA supports C++, Compute Unified Device Architecture (CUDA, [43]) for Graphics Processing Units (GPUs), Python, Perl and R for plugin development. A pipeline can be set up in the user layer using plugins that are heterogenous in their underlying implementation language, allowing users to have installed only the plugins and tools required for their particular task. We distribute PLUMA open source under the GNU General Public License (GPL), version 3.

1.1 Useful Features

1.1.1 Compatibility with a Wide Range of Languages

The computational back end of PLUMA has been written in C++ and is a simple, lightweight package that loads and runs plugins. However, plugin stages can be built in one of many possible languages. Currently, PLUMA supports plugins for both the CPU and GPU. On the CPU side they can be compiled using C/C++
or scripted using Python, Perl or R. For the GPU side, PLUMA supports CUDA. Because of the uniform back end that executes these plugins, a user need not worry about the particular implementation details of the plugin including its language, but can simply download the plugin and include it in their pipeline. Since plugins are dynamically loaded a user can just download and install the plugins they need. For example, a user may not have an NVIDIA graphics card, but they can still run any pipeline that uses CPU plugins. If they do not have R installed, they can still use C++ and/or Python plugins. The goal of PLUMA is thus to maximize both flexibility and extensibility in terms of both language and feature integration.

1.1.2 Minimal External Dependencies

As a result of this flexibility and the tool being lightweight, PLUMA requires only one external dependency for installation. This tool is SCons [30], which is available open-source and is a cross-platform compilation tool implemented in Python. The tool is easy to download and run through a single command `scons`.

1.1.3 Plugin Generator

A user may wish to integrate a standalone tool (i.e., Mothur [54]) as an inner stage of a larger pipeline. PLUMA includes a PluginGenerator module that can be used for this purpose. The PluginGenerator will produce a C++ wrapper plugin for the desired package, which can in turn be compiled and dynamically loaded as a stage in any general pipeline.

1.1.4 Log Files

Log files provide a useful method for tracking pipeline progress, particularly when timestamped with a date and a time. PLUMA outputs log files automatically for each pipeline execution, sending them to a file conveniently assigned a name that includes the date and time. Particularly for a pipeline that contains many stages, this can be a convenient way to determine a point of failure, or an intermediate interesting result [52]. Either can work with PLUMA’s Restart feature (described next), to start a pipeline from a middle point.

1.1.5 Restarting

Users can restart a pipeline from a specific stage, as opposed to the first stage, without having to change the configuration file. This is convenient if for example a log file (described above) displayed an error message at a particular stage of the pipeline, as the pipeline can be started from the erroneous stage as opposed to having to rerun from the beginning. Depending on the computational intensity of each stage, this could save a great deal of execution time.
Chapter 2

Availability and Installation of PLUMA

2.1 How to Download PLUMA

The download site for PLUMA is hosted by the Bioinformatics Research Group (BioRG) at Florida International University: http://biorg.cis.fiu.edu/pluma. Source code is available on this site as a tarball. An agreement to a Non-Exclusive, Non-Commercial Use License is required (this exact license can be found in the appendix of this user guide). We also make available a plugin pool of dynamically loadable plugin libraries implemented in various languages, as well as example data sets and configuration files.

2.2 Compiling PLUMA

PLUMA uses the SCons (http://www.scons.org) open source software construction tool to compile its back end. Please download and install SCons before compiling PLUMA. Once SCons is installed, the steps to compile PLUMA are:

1. Change to the main pluma directory.

2. Run the command "scons /". If you run only "scons /" and pass no flags, PLUMA will assume you have tools installed for all supported languages. At a minimum, PLUMA will assume that you have C++ and Python installed (these are necessary anyway to compile the back end and run scons, respectively). For the other three supported languages (CUDA, R, and Perl) you can turn off their compilation by passing flags cuda=0, r=0 and perl=0, respectively. This information is also outlined in the README file inside the PLUMA root directory.

2.2.1 Environment Variables

If your installations for R and/or Perl are not in default locations, you may need to specify these as environment variables. Below we provide a set of environment variables that PLUMA uses when determining the location of these libraries.

<table>
<thead>
<tr>
<th>Environment Variable</th>
<th>Description</th>
<th>Default</th>
</tr>
</thead>
<tbody>
<tr>
<td>RHOME</td>
<td>R root directory</td>
<td>/usr/share/R</td>
</tr>
<tr>
<td>RSITELIBHOME</td>
<td>Root directory of site-library directory for R</td>
<td>/usr/local/lib/R/site-library</td>
</tr>
<tr>
<td>PERLHOME</td>
<td>Perl root directory</td>
<td>/usr/</td>
</tr>
<tr>
<td>PTHREADHOME</td>
<td>PThread root directory</td>
<td>/usr/local/</td>
</tr>
</tbody>
</table>
Running `scons` compiles the PLUMA back end and all prepackaged plugins in the `plugins/` directory. Repeated runs of `scons` will only recompile those portions of the code that changed. By running `scons -c`, all object files and dynamically loaded libraries will clear recursively throughout the directory tree.

After everything has compiled successfully, the user may run PLUMA from the root directory. This is described in the next chapter.

### 2.3 Documentation

All available documentation for PLUMA can be downloaded from:

http://biorg.cs.fiu.edu/pluma/
Chapter 3

Getting Started

In this chapter, we introduce the commands needed to run PLUMA. These include the command line formats on UNIX-based machines, followed by a description of the configuration file formats. In the next section we show example configuration files that are included in the PLUMA installation, to help illustrate concepts.

3.1 Command Line

The application file for the PLUMA package is conveniently named pluma. To run the application, type pluma at a UNIX prompt followed by a configuration file and, optionally, a restart point. Thus the PLUMA execution command takes the following format:

```
./pluma (config file) (optional restart point)
```

The second argument can be an absolute or relative path to the configuration file. The restart point must be a valid plugin that has been specified in the configuration file provided in the second argument. If you provide a restart point, the pipeline will start from the stage specified by that plugin.

Below we show three example PLUMA executions, along with their meanings, assuming PLUMA has been installed in the location `/home/johndoe/` and the file `/home/johndoe/examples/myconfig.txt` is our configuration file with three plugins `Stage1`, `Stage2` and `Stage3`.

<table>
<thead>
<tr>
<th>Command</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><code>. / pluma /home/johndoe/examples/myconfig.txt</code></td>
<td>Run using the configuration file <code>/home/johndoe/examples/myconfig.txt</code></td>
</tr>
<tr>
<td><code>. / pluma examples/myconfig.txt</code></td>
<td>Same</td>
</tr>
<tr>
<td><code>. / pluma examples/myconfig.txt Stage1</code></td>
<td>Same</td>
</tr>
<tr>
<td><code>. / pluma examples/myconfig.txt Stage2</code></td>
<td>Same, but start with second stage</td>
</tr>
<tr>
<td><code>. / pluma examples/myconfig.txt Stage3</code></td>
<td>Same, but only run last stage</td>
</tr>
</tbody>
</table>

3.1.1 Plugin Location

The directory containing the plugins for PLUMA is assumed to be the directory of the `pluma` executable, followed by `plugins/`. So for example the above case would assume all plugins are in the folder `/home/johndoe/plugins`, which would be the case for all plugins that come with the PLUMA package. You can also set this location through an environment variable called `PLUMA_PLUGIN_PATH`. This can be used to load plugins from multiple directories, separated by the colon `:` character:
export PLUMA_PLUGIN_PATH=/usr/lib/plugins:/home/johndoe/johnsplugins

Setting this variable would load plugins from the PLUMA root directory, followed by /usr/lib/plugins, followed by /home/johndoe/johnsplugins. If two plugins in two different folders have the same name, PLUMA will use the second plugin.

### 3.1.2 Help Option

A user may also type one of the following two possibilities at a UNIX prompt for help with the PLUMA command line:

1. `./pluma` (with no arguments)
2. `./pluma help`

### 3.1.3 Version Option

A user may provide the argument `version` to get the release number of PLUMA that they are running:

1. `./pluma version`

### 3.1.4 Plugins Option

To get a list of all currently installed plugins, a user can provide the `plugins` option to `pluma`:

1. `./pluma plugins`

### 3.2 Configuration File

The configuration file is the only required command-line input to the PLUMA executable. This section discusses the structure of the PLUMA configuration file. In addition for guidance, PLUMA has a set of example configuration files already available in the `examples/` directory. We provide one of these here, which runs a complete metagenomics analysis pipeline (`FullPipeline`):

```plaintext
# Metagenomics analysis pipeline
Prefix data/Stability
Plugin Mothur inputfile Stability.mothur outputfile none
Plugin CountTableProcessing inputfile stability.trim.abund.pick.an.unique_list outputfile input.Stability.csv
Plugin CSVNormalize inputfile input.Stability.csv outputfile input.Stability.normalized.csv
Plugin Correlation inputfile input.Stability.normalized.csv outputfile correlations.pvalued.csv
Plugin CSVPad inputfile correlations.pvalued.csv outputfile Stability.pvalued.csv
Plugin GPUATria inputfile Stability.pvalued.csv outputfile Stability.pvalued.ATria.noa
# Plugin ATRia inputfile Stability.pvalued.csv outputfile Stability.pvalued.ATria.noa
Plugin CSV2GML inputfile Stability.pvalued.csv outputfile Stability.pvalued.gml
Plugin Cytoscape inputfile Stability.pvalued.gml outputfile none
```
This particular pipeline is eight stages long. As can be seen, it is quite easy to assemble and is composed of three different components: comments, prefixes, and plugins. We discuss each of these now.

### 3.2.1 Comments

PLUMA will ignore all characters on a line that follow the pound (#) sign. Comments are useful for writing well-documented configuration files, or also to quickly swap out a plugin and test a different one. For example in the case above we included a plugin for the *Ablatio Triadum* (ATria, [13]) algorithm, but then implemented a more efficient version on the GPU. To test for accuracy and performance improvement, we could simply comment out the CPU version of ATria and add the GPU version.

### 3.2.2 Prefix

The **Prefix** keyword allows you to set a relative or absolute path to use for data files (input and output). There will often be situations where you will use multiple input and output files that are in the same directory. This option allows you to specify that directory once and then assume it, as opposed to having to retype it for every `inputfile` and `outputfile` in the configuration file. In the case above for example, all of our data files were contained in `data/Stability`, so we specified that as the first line of the PLUMA configuration file. You are allowed to use multiple lines that start with **Prefix**, if at a later stage you want the prefix to change. For a given `inputfile` or `outputfile`, PLUMA will always append the last **Prefix** specified (or none if one was never specified).

### 3.2.3 Plugins

The core component of the PLUMA configuration file is a sequential collection of unique plugin identifiers (one for each stage of the pipeline), their input files, and their output files:

```
Plugin plugin1 inputfile inputfilename1 outputfile outputfilename1
Plugin plugin2 inputfile inputfilename2 outputfile outputfilename2
Plugin plugin3 inputfile inputfilename3 outputfile outputfilename3
...
```

The plugin identifiers (*plugin1*, *plugin2*, *plugin3*, etc.) must refer to names of plugins installed within the `PLUMA_PLUGIN_PATH` or the `plugins/` folder in the PLUMA source tree. Output files will be written by their respective plugins, and input files will be read. If a particular plugin does not read and/or write a file, specify `none` as the value of `inputfile` and/or `outputfile`, respectively. Input files must exist at the point when a plugin is executed, but not necessarily at the start of the pipeline execution. Since the configuration file is sequential, an input file could be an output file of a previous plugin. Note while it will often be the case for the input file of a particular plugin to be the same as the output file name of its immediately preceding plugin, that need not necessarily be the case. In our above example, both **GPUATria** (Stage 6) and **CSV2GML** (Stage 7) took the same file `input.Stability.csv` as input, which was produced as an output of **CSVPad** (Stage 5).
3.2.4 PLUMA prepackaged plugins

Upon installing PLUMA, you receive a set of prepackaged plugins along with some example configuration files that use them. The plugins are contained within the plugins/ subdirectory of the PLUMA source tree, and the configuration files are under examples/. Input and output files referenced in the configuration files will be in the data/ subdirectory.

Below we provide a description of each of these plugins, that includes their plugin identifier, source code language, and input and output file formats. Note that many of the plugins convert between file formats, which can be useful for intermediate stages of a PLUMA pipeline.
<table>
<thead>
<tr>
<th>Plugin ID</th>
<th>Language</th>
<th>Description</th>
<th>Input Format</th>
<th>Output Format</th>
</tr>
</thead>
<tbody>
<tr>
<td>AffinityPropagation</td>
<td>Python</td>
<td>Runs Affinity Propagation [20], an algorithm for clustering that uses message-passing between nodes.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>ATRia</td>
<td>C++</td>
<td>Runs the Albatio Triadum (ATria) algorithm for centrality (CPU version).</td>
<td>CSV</td>
<td>NOA</td>
</tr>
<tr>
<td>AutoCorrelation</td>
<td>R</td>
<td>Computes the autocorrelation value of a time series variable or set of variables.</td>
<td>TXT</td>
<td>TXT</td>
</tr>
<tr>
<td>Binomial</td>
<td>R</td>
<td>Distance function for a network that uses Binomial Deviance [35].</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Bray</td>
<td>R</td>
<td>Bray-Curtis [8] distance function.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>CalcMeanStd</td>
<td>Python</td>
<td>Calculate and print the mean and standard deviation of a dataset.</td>
<td>NOA</td>
<td>none</td>
</tr>
<tr>
<td>Canberra</td>
<td>R</td>
<td>Canberra [33] distance function.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Chao</td>
<td>R</td>
<td>Chao's Method [10].</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Classify</td>
<td>Python</td>
<td>Properly classify bacterial OTUs at the lowest level in the phylogenetic tree.</td>
<td>NOA</td>
<td>NOA</td>
</tr>
<tr>
<td>ClusterizeCSV2NOA</td>
<td>Python</td>
<td>Takes a clusterized CSV file and converts it to NOA format. This in turn can be used by Cytoscape to color nodes by cluster.</td>
<td>CSV</td>
<td>NOA</td>
</tr>
<tr>
<td>Clusterize</td>
<td>Python</td>
<td>Takes a network and a set of clusters, and output a modifies adjacency matrix that includes only edges between neighbors in the same cluster.</td>
<td>prefix</td>
<td>CSV</td>
</tr>
<tr>
<td>CountTableProcessing</td>
<td>R</td>
<td>Takes a set of abundances and OTUs from Mothur, and computes an abundance matrix.</td>
<td>prefix</td>
<td>CSV</td>
</tr>
<tr>
<td>CrossCorrelation</td>
<td>R</td>
<td>Cross-correlate two univariate time series.</td>
<td>prefix</td>
<td>TXT</td>
</tr>
<tr>
<td>CSV2GML</td>
<td>Python</td>
<td>Converts a CSV file to GML.</td>
<td>CSV</td>
<td>GML</td>
</tr>
<tr>
<td>CSVMapRange</td>
<td>Python</td>
<td>Takes two files of abundances and maps the values of the first into the range of the second.</td>
<td>prefix</td>
<td>CSV</td>
</tr>
<tr>
<td>CSVNeg2Zero</td>
<td>Python</td>
<td>Remove all negative edges.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>CSVNormalize</td>
<td>Python</td>
<td>Takes a CSV file and normalizes its values across rows.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>CSVPad</td>
<td>Python</td>
<td>Pads the top row of a CSV file with a null string. Useful when reading as a table.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>CSVScale</td>
<td>Python</td>
<td>Sets zero elements to the minimum value, and rescales.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>CSVTranspose</td>
<td>Python</td>
<td>Takes a matrix and computes its transpose.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>CSVZero2Min</td>
<td>Python</td>
<td>Sets zero elements to the minimum value but does not rescale.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Cytoscape</td>
<td>C++</td>
<td>Runs Cytoscape [15] on the input network. Assumes Cytoscape is in your PATH.</td>
<td>GML</td>
<td>none</td>
</tr>
<tr>
<td>CytoViz</td>
<td>Perl</td>
<td>Scripted version of Cytoscape plugin.</td>
<td>GML</td>
<td>none</td>
</tr>
<tr>
<td>Degree</td>
<td>C++</td>
<td>Runs weighted degree [24] centrality.</td>
<td>CSV</td>
<td>NOA</td>
</tr>
<tr>
<td>Detrend</td>
<td>R</td>
<td>Least-squares fitting of time-series data.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Plugin ID</td>
<td>Language</td>
<td>Description</td>
<td>Input Format</td>
<td>Output Format</td>
</tr>
<tr>
<td>-------------</td>
<td>----------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Dickey-Fuller</td>
<td>R</td>
<td>Augmented Dickey-Fuller Test [53] for time-series data.</td>
<td>TXT</td>
<td>TXT</td>
</tr>
<tr>
<td>DistanceCorrelation</td>
<td>R</td>
<td>Distance Correlation Function [57].</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>EM</td>
<td>C++</td>
<td>K-Group Community Detection [41].</td>
<td>GML</td>
<td>TXT</td>
</tr>
<tr>
<td>Euclidean</td>
<td>R</td>
<td>Euclidean distance function.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Exponential</td>
<td>Python</td>
<td>Exponential centrality [7].</td>
<td>CSV</td>
<td>NOA</td>
</tr>
<tr>
<td>Gower</td>
<td>R</td>
<td>Computes Gower index.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>GPUATria</td>
<td>CUDA</td>
<td>Runs the Albatio Triadum algorithm for centrality (GPU version). Useful for large networks.</td>
<td>CSV</td>
<td>NOA</td>
</tr>
<tr>
<td>Horn</td>
<td>R</td>
<td>Horn’s overlap index.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>InverseSimpson</td>
<td>Python</td>
<td>Inverse Simpson diversity measure.</td>
<td>CSV</td>
<td>none</td>
</tr>
<tr>
<td>Kendall</td>
<td>R</td>
<td>Kendall correlation [28].</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Kulczynski</td>
<td>R</td>
<td>Kulczynski similarity [32].</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>LSA</td>
<td>Python</td>
<td>Local Similarity Analysis [51].</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>MakeClique</td>
<td>Python</td>
<td>Create a network with a certain number of cliques.</td>
<td>TXT</td>
<td>CSV</td>
</tr>
<tr>
<td>MakeSynthetic</td>
<td>Python</td>
<td>Create a synthetic network.</td>
<td>TXT</td>
<td>CSV</td>
</tr>
<tr>
<td>Manhattan</td>
<td>R</td>
<td>Manhattan distance.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Map2Positive</td>
<td>Python</td>
<td>Maps a [-1,1] range to [0,2], useful for correlations and visualization.</td>
<td>CSV</td>
<td>EDA</td>
</tr>
<tr>
<td>MappedWeight</td>
<td>Python</td>
<td>Sets negative edges to zero and produces an edge file for Cytoscape (this allows the original weights to be kept).</td>
<td>CSV</td>
<td>EDA</td>
</tr>
<tr>
<td>MCL</td>
<td>R</td>
<td>Runs Markov clustering [59].</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>MetaBAT</td>
<td>C++</td>
<td>Runs the MetaBAT [26] software, if installed.</td>
<td>TXT</td>
<td>prefix</td>
</tr>
<tr>
<td>MIC</td>
<td>R</td>
<td>Computes maximal information coefficient [49].</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>ModularityMaximization</td>
<td>Python</td>
<td>Detects communities using the Modularity Maximization [40] algorithm.</td>
<td>prefix</td>
<td>none</td>
</tr>
<tr>
<td>Morisita</td>
<td>R</td>
<td>Computes Morisita overlap [37].</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Mothur</td>
<td>C++</td>
<td>Runs the Mothur software, if installed.</td>
<td>MOTHUR</td>
<td>none</td>
</tr>
<tr>
<td>Mountford</td>
<td>R</td>
<td>Computes the Mountford Dissimilarity index [38].</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>NetworkViz</td>
<td>Python</td>
<td>Takes a prefix for a CSV and cluster CSV file, and generates appropriate files for Cytoscape visualization.</td>
<td>prefix</td>
<td>COM</td>
</tr>
<tr>
<td>NormScoreTransform</td>
<td>Python</td>
<td>Normalizes across columns.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>PageRank</td>
<td>Python</td>
<td>Runs Google’s PageRank centrality algorithm.</td>
<td>CSV</td>
<td>NOA</td>
</tr>
<tr>
<td>PCL2CSV</td>
<td>Python</td>
<td>Converts a PCL file to CSV.</td>
<td>PCL</td>
<td>CSV</td>
</tr>
<tr>
<td>Pearson</td>
<td>R</td>
<td>Pearson [46] correlation.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Prestige</td>
<td>C++</td>
<td>Calculates Katz prestige [27] centrality.</td>
<td>CSV</td>
<td>NOA</td>
</tr>
<tr>
<td>PyATria</td>
<td>Python</td>
<td>Scripted version of Albatio Triadum.</td>
<td>CSV</td>
<td>NOA</td>
</tr>
<tr>
<td>QGraph</td>
<td>R</td>
<td>Visualizes a network using the R QGraph library.</td>
<td>prefix</td>
<td>PNG</td>
</tr>
<tr>
<td>Raup</td>
<td>R</td>
<td>Runs the Raup-Crick [48] dissimilarity algorithm.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Plugin ID</td>
<td>Language</td>
<td>Description</td>
<td>Input Format</td>
<td>Output Format</td>
</tr>
<tr>
<td>---------------</td>
<td>----------</td>
<td>------------------------------------------------------------------------------</td>
<td>--------------</td>
<td>---------------</td>
</tr>
<tr>
<td>ReactionPathway</td>
<td>Python</td>
<td>Takes a set of input files and determines statistics based on how often two metabolites are on the same reaction pathway, and in the same cluster.</td>
<td>prefix</td>
<td>TXT</td>
</tr>
<tr>
<td>RemoveNegative</td>
<td>Python</td>
<td>Takes a network and makes all negative edges positive.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>SIMLR</td>
<td>R</td>
<td>Similarity Learning algorithm for clustering [60].</td>
<td>prefix</td>
<td>prefix</td>
</tr>
<tr>
<td>Spearman</td>
<td>R</td>
<td>Spearman [56] correlation.</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Spectral</td>
<td>Python</td>
<td>Runs spectral clustering [4].</td>
<td>CSV</td>
<td>CSV</td>
</tr>
<tr>
<td>Variance</td>
<td>Python</td>
<td>Takes a CSV file and computes variance in weights across rows.</td>
<td>CSV</td>
<td>TXT</td>
</tr>
</tbody>
</table>

PLUMA prepackaged plugins use the following file formats:

1. **Comma-Separated Value (CSV)**: A tabulated data format consisting of a header followed by rows, with each data cell separated by commas. Microsoft Excel commonly uses CSV files.

2. **Graph Modeling Language (GML)**: Textual network format with modules for nodes and edges, used by Gephi [5] and Cytoscape.

3. **Node Attribute File (NOA)**: A simple table of node names and attribute values, accepted by Cytoscape.

4. **Edge Attribute File (EDA)**: A table of edge names and attribute values, also accepted by Cytoscape.

5. **Pre-CLustered File (PCL)**: Describes a gene (often regulatory) network, used by the Stanford Microarray Database [16].


7. **Mothur Program (MOTHUR)**: A program written in Mothur’s domain-specific language.

8. **Portable Network Graphics (PNG)**: Image format.

9. **Cytoscape COMmands (COM)**: Cytoscape scripting language.

10. **prefix**: Indicates that the plugin will be reading/writing multiple files, in this situation just accept the prefix.

    New PLUMA plugin extensions need not use one of these file formats. The important matter is that whenever you use a plugin, a file in its accepted format must either exist or be produced by an earlier pipeline stage.
Chapter 4

Extending PLUMA

Since as we mentioned our primary goal in developing PLUMA is infinite extensibility, its most important component does not lie in the features that exist, but those that will exist in the future as new plugins. We thus now provide an outline on how to develop a new plugin for PLUMA.

Plugins can be built using existing tools or even existing plugins, or be constructed entirely from scratch in your language of choice. Our PluginGenerator module can be used if you want to include an existing, working software tool as part of the PLUMA pipeline. We describe that first. Next, we give an outline on how to develop a plugin in all languages supported by PLUMA. Finally, we illustrate how to take components of an existing plugin and use them as part of a new one. Examples of all of these exist in the PLUMA prepackaged plugins, and we will refer to those examples as we describe these processes below.

4.1 Generating a New Plugin for An Existing Software Package

In many situations, you may have an existing tool that is already fully developed and tested, and you would like to include that tool as a part of your PLUMA pipeline. PLUMA includes a plugin generator tool for this purpose, which is available in the PluginGenerator/ subdirectory. The generated plugin will be in C++, will assume that this existing tool is within your system PATH, and will be automatically placed in the plugins/ subdirectory of the PLUMA source tree. The advantage of placing it here is that no modifications to any PLUMA compile scripts will be required. These scripts automatically check for new or modified plugins within plugins/, since that is the default location for all PLUMA plugins. We chose C++ as the language for the generated plugin, since it is the same language as the PLUMA back end and thus will require no additional language tools to be installed.

The plugin generator is automatically compiled as a part of the PLUMA package when running SCons. To run the plugin generator, change to the PluginGenerator/ subdirectory.

4.1.1 Option 1: Single Command

For a package that accepts no or predefined command line arguments, the command to generate the plugin is straightforward:

```
./generate plugin_name command_to_run
```

Where `plugin_name` is the name of the new PLUMA plugin, and `command_to_run` is the command that should be used to run the software. Be sure that, unless you intend to replace an existing plugin in the plugins/ subdirectory, that `plugin_name` does not name an existing plugin. Otherwise the existing plugin that shares the same name will be automatically overwritten. As an example, to generate a plugin that runs Mothur with no command line arguments, you can run:
since `mothur` is the name of the executable file for Mothur.

### 4.1.2 Option 2: Input and Output File

Most software tools (including Mothur) will take some type of input parameter(s), however. In a simple case, the only dependencies can be the `inputfile` and `outputfile` that are specified in the configuration file. Recall that our earlier configuration file specification for our `Mothur` plugin looked like this:

```
Plugin Mothur inputfile Stability.mothur outputfile none
```

For the generator, this `inputfile` keyword can also act as a placeholder for the argument that is specified in the configuration file. For example, this `Mothur` plugin accepts one input file and produces no output. We generated this plugin with the command:

```
./generate Mothur mothur inputfile
```

So that if the user provided `Stability.mothur` as the `inputfile` in the configuration file, this will automatically be plugged in as `inputfile` here, and the command "`mothur Stability.mothur`" will be executed by this plugin. Thus the `PluginGenerator` can automatically produce plugins that can be customized by the user at runtime.

### 4.1.3 Option 3: Fully Customized

Other software tools such as Cytoscape will take multiple input parameters. Since PLUMA plugins in general can only accept a single input file, in this situation the generated plugin will accept one plaintext file, in the following format:

```
keyword1 value1
keyword2 value2
keyword3 value3
... 
```

Where the keyword values are specified in the command to `generate`. For example, our Cytoscape plugin was generated using the following command:

```
./generate Cytoscape cytoscape -N networkfile -s sessionfile
```

We also include a file in `data/Stability` called `Stability.visualization.txt`, with the following contents:

```
networkfile Stability.pvalued.gml
sessionfile default.cys
```

By providing `Stability.visualization.txt` as the `inputfile` argument to the Cytoscape plugin in the PLUMA configuration file, this will run the following command:

```
cytoscape -N Stability.pvalued.gml -s default.cys
```
Thus the user-specified values in the input plain text file automatically get plugged in to their corresponding keyword in the execution command. Note that command line arguments that start with a dash (\texttt{-}) are copied directly. The output file can still be used as before in the command line. If \texttt{inputfile} is detected in the arguments to \texttt{generate} the PluginGenerator will automatically assume Option 2, otherwise it will use Option 3. Therefore you must make sure to not use \texttt{inputfile} as a keyword if you desire Option 3.

### 4.2 Building a New Plugin From Scratch

We now describe how to build a new plugin entirely from scratch, using one of the supported languages of PLuMA. These languages have a wide variety of syntax and semantics, some are object-oriented and some are not, some are compiled and some are scripted, some run on the CPU and some run on the GPU. As one can thus imagine, the process of setting these up will vary by the language, however it is important to remember that from the perspective of running PLuMA, nothing will be different. When specifying a plugin in the configuration file, you do not need to know in which language it is written, nor do you need to worry about parsing the configuration file differently depending on the language. You only need to be concerned with writing the functionality of the plugin itself.

One programming unit that all of these languages do have in common is procedures. There are three procedures that you will need to setup, independent of the language you choose:

1. An \texttt{input()} procedure that accepts the name of an input file, reads it, and performs initialization.
2. A \texttt{run()} procedure that accepts zero parameters, and runs the plugin.
3. An \texttt{output()} procedure that accepts the name of an output file, finalizes the plugin, and writes the file.

We now review the five supported languages, examples of how to write those procedures in the respective languages, and any particular nuances that you need to follow given your language of choice. With respect to extensibility however, no one language has advantages over the other in terms of ease. We included this flexibility to accomodate a wide range of user preferences with respect to programming languages.

#### 4.2.1 C++

For our C++ example we use the \texttt{ATria} plugin, which takes as input a signed and weighted network in CSV format, runs the Ablatio Triadum algorithm for centrality (importance), and outputs a list of the most important nodes in the network in NOA format. This latter format makes it convenient to import the file into Cytoscape and perform a useful visualization such as coloring the nodes in the network by centrality value.

When constructing a new plugin, you must include it inside a subdirectory of either the default PLuMA plugins directory or another directory within the \texttt{PLUMA_PLUGIN_PATH}. The subdirectory should uniquely define the plugin, and your C++ source files should use that name followed by \texttt{Plugin}. This also should be the name of your new C++ class, which will inherit from the parent class \texttt{Plugin}. As an example, assuming we are currently in the \texttt{plugins} directory - we would first make a directory \texttt{ATria} to hold all source files. Then, we can setup the header file for this plugin \texttt{ATria/ATriaPlugin.h} as shown in Program 1.

As is typical in a C++ header file, preprocessor macros (\#ifndef, \#define and \#endif) should be used to avoid including the file multiple times. New C++ plugins must include two files from PLuMA: \texttt{Plugin.h} and \texttt{PluginProxy.h}, as well as any other files that need to be included (i.e. from the Standard Template Library [39], we included its \texttt{string} class above). Our new class will be named \texttt{ATriaPlugin}, which as mentioned inherits from \texttt{Plugin}. Within the class, the only requirements are that there must be three procedures: \texttt{input}, \texttt{run}, and \texttt{output}. The \texttt{input} and \texttt{output} procedures must accept one \texttt{std::string} parameter for the input and output file, respectively. Note these are required \textit{even} if they do not do anything for this particular plugin, you just can leave their definitions empty. From there, you can feel free to include any other necessary member variables or procedures to accomplish your particular task. In Program 1, we have variables for the network (an array of \texttt{float}) and bacteria.
#ifndef ATRIAPLUGIN_H
#define ATRIAPLUGIN_H

#include "Plugin.h"
#include "PluginProxy.h"
#include <string>

// Other necessary includes...

class ATriaPlugin : public Plugin {
    public:
    // These are required
    void input(std::string file);
    void run();
    void output(std::string file);

    // Other member procedures...

    private:
    float* OrigGraph;
    std::string* bacteria;
    // Other member variables...
};

#endif
(the names of nodes in this network, an array of std::string). This is so named because ATria was originally tested on bacterial co-occurrence networks [18].

The corresponding source file, ATria/ATriaPlugin.cpp is setup as shown in Program 2.

**Program 2** ATria/ATriaPlugin.cpp

```cpp
#include "PluginManager.h"
#include "ATriaPlugin.h"

void ATriaPlugin::input(std::string file) {
    // Read file, and initialize member variables...
}

void ATriaPlugin::run() {
    // Run the algorithm...
}

void ATriaPlugin::output(std::string file) {
    // Perform any final operations, and write file...
}

// Other member procedure definitions...

// Required, connects the plugin to the PluMA back end
PluginProxy<ATriaPlugin> ATriaPluginProxy
    = PluginProxy<ATriaPlugin>("ATria", PluginManager::getInstance());
```

There are two necessary includes at the top of the file. PluginManager.h is required because at the end of the file we make use of PluMA’s PluginManager to interface the new plugin with the PluMA back end. Here we create a PluginProxy which uses the Proxy [3] design pattern to register the new plugin with the PluginManager. As arguments to its constructor, it accepts the unique plugin name ATria as a string, along with the PluginManager. This will correspond to its name in a PluMA configuration file. The other required include is standard C++ practice, the corresponding header file for this class. Any other necessary includes can then follow.

The source file now must define input, run and output. We provide these three procedures for convenience in terms of separating functionality. The one requirement is that if your plugin will require an inputfile in the PluMA configuration file it must be read in input, and similarly an outputfile must be written in output. From there as the new plugin developer you have control over what functionality goes where, but the convention is to initialize member variables and prepare to run the new algorithm in input, actually run the algorithm in run, and perform any final operations before writing the outputfile in output. As an example, our implementation of ATriaPlugin reads the CSV file and populates the graph and bacteria arrays in input, executes the centrality algorithm in run, and sorts the list of nodes by centrality value before writing the NOA file in output.

The SCons scripts of PluMA will automatically detect and compile the new plugin as a shared object (so) file if it is stored in the plugins/subdirectory of the PluMA root. We do not compile everything in the PLUMA_PLUGIN_PATH for now, to avoid potentially accessing installation folders for which a user may not have permission. Shared objects are loaded at runtime, which will help to keep both the executable and execution environment of PluMA lightweight.
4.2.2 CUDA

We provide CUDA as an option for constructing a Graphics Processing Unit (GPU) plugin that can run on an NVIDIA graphics card. The large number of cores and multithreading within each core have made the GPU a solid option for many bioinformatics applications [9]. As an example, applying the GPU to ATria yielded an order of magnitude improvement in speed for large networks [12], allowing us to complete analysis of a 3000-node fruit fly network in a few hours instead of a few days.

CUDA is an extension of C and recent additions to the NVIDIA compiler allow GPU functionality to be invoked from C++ classes, so building a CUDA plugin will not be much different from building a C++ plugin. The first difference is in the filename; while the header file can still end in a .h extension the source file must now end in .cu. This extension is recognizable by the NVIDIA compiler, and is what tells the PLuMA SCons scripts to use this compiler as opposed to the standard GNU compiler.

The second difference is that while GPU functionality can be invoked from a C++ class, it cannot be contained in a member procedure of a C++ class. Rather, it must be implemented as a standalone kernel procedure where all parameters and variables are assumed to be allocated on the GPU. As an example, see our new header file for our plugin GPUATria in Program 3.

Note that the preprocessor directives, required includes, and C++ class template are the same. However, at the bottom of the file we include two GPU kernel procedure headers. In CUDA these always begin with _global_ and accept parameters that have been allocated on the GPU. In our implementation we run a GPU version of Floyd-Warshall [19], [44] which runs the all-pairs shortest path algorithm, and then another kernel procedure to compute centrality, which we call pay as ATria was economically-based. Any number of GPU kernels can be declared outside of the C++ class.

The source file GPUATria/GPUATriaPlugin.cu now must include, in addition to the member procedure definitions, the kernel procedure definitions. These then will be called from the member procedures. We show this template in Program 4. Otherwise, there is nothing different from defining a C++ plugin.

Since GPU parallelism will be used for computationally expensive tasks it is likely that you will invoke your GPU kernels from the run procedure, though this is not required. CUDA procedures are called with a certain number of thread blocks and threads per block, which are provided as arguments to the kernel within the triple carat <<< and >>> operators. Their values can have critical effects on the efficiency of GPU code [34]. For more information, please view the CUDA Programmer’s Guide [42].

As with C++ plugins, the SCons scripts of PLuMA will compile any CUDA plugins as shared objects.

4.2.3 Python

The major difference with the rest of the PLuMA supported languages is that they are scripted. This difference is critical in terms of system architecture. First, any scripted plugins by definition will not require compilation at all, and thus there is no mechanism built into the PLuMA SCons scripts to detect their presence (though they still will be detected at runtime). Another big difference is that you can contain the entire plugin within one file. You can still use multiple files if you choose (perhaps you want to borrow an existing procedure or module from someplace else), however the functionality of the plugin should all be placed in the same file.

As our Python example, we will use the PageRank plugin - which runs Google’s PageRank algorithm and like ATria computes a centrality score for every node in the network. The location and name of this file should follow the same conventions up to this point - from a location in the PLUAMA_PLUGIN_PATH create a new directory that corresponds to the name of the new plugin (in this case, PageRank) and use this to name the file followed by Plugin (in this case, PageRank/PageRankPlugin.py). Once again because there is no compilation, we no longer have a need to create a proxy to link the new plugin module with the back end. Program 5 shows our template for PageRankPlugin.

This particular plugin is a good example of some advantages of scripting. Our implementation uses the NetworkX [23] libraries for running PageRank, PythonDS [36] to construct the graph, and Numerical Python [58] to perform useful and efficient calculations. All of these are easily importable at the top of the file. We follow these with global variables, and any procedure definitions (here we define a procedure that takes a CSV file and produces a graph that NetworkX accepts).
Program 3 GPUATria/GPUATriaPlugin.h

```c
#include <string>
// Other necessary includes...

class GPUATriaPlugin : public Plugin {
    // These are required
    void input(std::string file);
    void run();
    void output(std::string file);

    // Other member procedures...

    private:
        float* OrigGraph;
        std::string* bacteria;
        // Other member variables...
};

// Other GPU kernels...
```

```c
global void _GPU_Floyd_kernel(int k, float* G, int N);
```
Program 4  

```cpp
#include "PluginManager.h"
#include "GPUATriaPlugin.h"
// Other necessary includes...

void GPUATriaPlugin::input(std::string file) {
    // Read file, and initialize member variables...
}

void GPUATriaPlugin::run() {
    // Run part of the algorithm...

    // At some point call first kernel
    _GPU_Floyd_kernel<<<dimGrid, BLOCK_SIZE>>>(k, dG, N);

    // ...

    // At some point call second kernel
    _GPU_Pay_kernel<<<numblocks, BLOCK_SIZE>>>(dG, dPay, (N/2));

    // Finish the algorithm....
}

void GPUATriaPlugin::output(std::string file) {
    // Perform any final operations, and write file...
}

// Other member procedure definitions...

__global__ void _GPU_Pay_kernel(float* D, float* P, int N) {
    // GPU code...
}

__global__ void _GPU_Floyd_kernel(int k, float *G, int N)
{
    // GPU code...
}

// Other kernel procedure definitions...

// Required, connects the plugin to the PluMA back end
PluginProxy<GPUATriaPlugin> GPUATriaPluginProxy
    = PluginProxy<GPUATriaPlugin>("GPUATria", PluginManager::getInstance());
```
import numpy
import networkx
from pythonods.graphs import PriorityQueue, Graph, Vertex
# Any other imports...

numdiff = 0
ALPHA=0.5

def buildNetworkXGraph(myfile):
    # Read myfile, build and return graph

# Any other global variables or procedures...

class PageRankPlugin:
    def input(self, file):
        self.bacteria, self.graph = buildNetworkXGraph(file)
    def run(self):
        self.U = networkx.pagerank(self.graph, alpha=ALPHA, max_iter=100)
    def output(self, file):
        UG = []
        for key in self.U:
            UG.append((self.U[key], key))
        UG.sort()
        UG.reverse()

        # Write file...
Like C++, Python is object-oriented. Thus similarly, we create a class `PageRankPlugin` and define three procedures `input`, `run`, and `output`. In `input`, we call our procedure which reads the input CSV file and builds the graph. In `run` we execute the PageRank algorithm, which consists of a call to the NetworkX functionality. Finally in `output` we sort the centrality values produced by PageRank and output the NOA file for Cytoscape. Note that member procedures in Python must include `self` as their first parameter, and member variables can be created on the fly by appending `self` to the front of their identifiers. There are thus no declarations of variables or plugins in Python, which is also the case in most scripting languages.

### 4.2.4 R

The final two scripting languages, R and Perl, are not object-oriented by default. However, there are importable packages available in both cases that facilitate object-oriented programming. To uphold compatibility with the standalone tools for each language, we do not use classes to represent plugins in either of these options.

In the case of R we use the same naming scheme, but instead of defining three member procedures `input`, `run` and `output` they will be defined as standalone procedures. Program 6 shows our R implementation of a Correlation plugin, which takes as input a CSV file of abundances and outputs a CSV file of correlations.

The plugin file should start with any global variables. In our case we have a `p.value` that we use to threshold our correlations, and anything below this we assume a value of zero. Any external libraries that should be imported (in our case `Hmisc` will give us access to the R procedure `rcorr`) also should be placed at the top of the file. We then define the three required procedures and as before, with `input` and `output` taking one parameter for their respective files. In our case the input procedure reads the CSV file of abundances and stores them in a matrix `pc`. The `run` procedure does some processing to `pc` (removing headers, converting to numbers) before computing correlations and thresholding them using our `p.value`. Finally, `output` writes its CSV file as a table of correlations. You can define other helper procedures in addition to these. Also since there is no longer a class interface, variables that need to be accessible across procedure calls must be global. In R, these are assigned using `<<-` instead of the standard `<-` for local variables. In our case, `pc` and `cn` (the graph and the headers) are global.

### 4.2.5 Perl

Building a plugin in Perl follows closely the steps to building a plugin in R, with three standalone procedures `input`, `run` and `output`. The naming strategies are also the same as those used when developing plugins for the other languages. We have developed a `CytoViz` plugin that takes an GML file and visualizes the corresponding network using Cytoscape, in Program 7.

This particular plugin will automatically launch Cytoscape assuming that its install directory is stored in the `CYTOSCAPE_HOME` environment variable. Note that in Perl, procedure parameters are not specified within parentheses but their values are automatically stored in the predefined array `@_`. Since this plugin is only launching Cytoscape, there is nothing to output and we simply return from that procedure. Global variables that should be accessible across procedures should be declared using the `my` keyword at the top of the file. In our case the sole global variable is the input file `$gmlfile`. In `run` we either launch Cytoscape using the `-N` flag to pass a network input file, or provide a message to the user to set `CYTOSCAPE_HOME` properly.

### 4.3 Building a New Plugin using Existing Plugins

In our Python section, we designed a plugin to implement Google’s PageRank algorithm for centrality. The standard PageRank algorithm works by simulating a random walk through a network. The walker always resides on a particular node in the network and upon every move has a probability $\alpha$ of going to a neighbor node, and a probability $1 - \alpha$ of teleporting to somewhere random in the network. Centrality values then depend on the amount of times a node is visited, which will be large for nodes with lots of connections and those connected to other central nodes. PageRank is an eigenvector-based approach, which iteratively solves a system of equations with the leading eigenvector converging to the vector of centrality values.
Program 6 Correlation/CorrelationPlugin.R

p_value <- 0.01;
# Any other global variables...

libs <- c('Hmisc');
# Any other libraries to import

# Import libraries
lapply(libs, require, character.only=T);

# Required
input <- function(inputfile) {
  pc <<- read.csv(inputfile, header = TRUE);
}

# Required
run <- function() {
  cn <<- colnames(pc);
  cn <<- cn[2:length(cn)];
  pc <<- pc[,c(-1)];
  pc <<- apply(pc, 1, as.numeric);
  pc <<- t(pc);
  correlations <<- rcorr(pc);
  pc <<- as.matrix(correlations$r);
  pc[is.na(pc)] <<- 0;
  empty <- c('');
  pc[which(correlations$P>p_value)] <<- 0;
}

# Required
output <- function(outputfile) {
  write.table(pc, file=outputfile, sep=' ', append=FALSE,
              row.names=unlist(cn), col.names=unlist(cn), na='');
}

# Other helper procedures...
Program 7 CytoViz/CytoVizPlugin.pl

my $gmlfile;
# Any other global variables ...

# Required
sub input {
    $gmlfile = @_[0];
    return;
}

# Required
sub run {
    $cytohome = $ENV{'CYTOSCAPE_HOME'};
    length($cytohome) != 0 or die 'Please set CYTOSCAPE_HOME\n';
    @args = ($cytohome . '/cytoscape.sh', '-N', $gmlfile);
    system(@args) == 0 or die 'system @args failed: $?\n';
    return;
}

# Required
sub output {
    return;
}
Later works have shown that the PageRank algorithm can be artificially biased, to favor nodes with certain properties over others. For example, one way of biasing PageRank is to use multiple values of $\alpha$. We could have a higher $\alpha$ value (call it $\alpha_1$) for neighboring nodes in the same cluster, and a lower value (call it $\alpha_2$) for neighboring nodes in different clusters. The probability of teleporting then becomes $1 - \alpha_1 - \alpha_2$. We have found such an approach to more clearly isolate cluster leaders in their centrality scores, as the walker will tend to stay in the same cluster for a longer period of time and thus visit its leader node more often.

We have already shown the PageRankPlugin that has been implemented in Python. We have another plugin ClusterizePlugin, which takes as input a network and associated cluster identifiers (one per node) and has a function inSameCluster() which returns true if the node pair are in the same cluster, and false otherwise. To build our new BiasedPageRankPlugin, we will borrow functionality from both plugins. Program 8 shows our Python implementation.

Program 8 BiasedPageRank/BiasedPageRankPlugin.py

```python
import numpy
import networkx as nx

import plugins.Clusterize.ClusterizePlugin
import plugins.PageRank.PageRankPlugin

def biasedpagerank(G, clusters, alpha1=0.5, alpha2=0.35, max_iter=100, 
                   tol=1.0e-8, nstart=None):
    # Biased Algorithm...
    # Use appropriate alpha value
    for nbr in W[n]:
        if (ClusterizePlugin.inSameCluster(n, nbr, clusters)):
            x[nbr]+=alpha1*xlast[n]*W[n][nbr]["weight"]
        else:
            x[nbr]+=alpha2*xlast[n]*W[n][nbr]["weight"]
    # Biased Algorithm...
```

```python
class BiasedPageRankPlugin(PageRankPlugin.PageRankPlugin):
    def input(self, filename):
        PageRankPlugin.input(self, filename + '.csv')
        self.clusters = ClusterizePlugin.readClusterFile(filename + '.clusters.csv')
    def run(self):
        self.U = biasedpagerank(self.graph, self.clusters, alpha1=0.5, 
                                alpha2=0.35, max_iter=100)
```

29
Note that we were able to import our ClusterizePlugin and PageRankPlugin to gain access to their functionality. We have our BiasedPageRankPlugin inherit from PageRankPlugin, since it works almost the same way but with a slight modification. This relationship follows the IS-A relationship that justifies using inheritance in object-oriented programming. The call to the inSameCluster function of the ClusterizePlugin enables us to choose the correct $\alpha$ value to use when updating the vector of centrality values computed by PageRank in our biasedpagerank procedure. We also can reuse the input procedure of the PageRank plugin when reading the CSV file (since it also reads a CSV file), and use the readClusterFile procedure of ClusterizePlugin to read the file of cluster identifiers for every node. The run procedure now calls the new biasedpagerank procedure, and the output procedure has no differences from that of the PageRankPlugin so we do not define a new one and use the inherited procedure.

This method of extending PLUMA will thus minimize the amount of time a user must spend reinventing the wheel, allowing them to focus specifically on their new ideas.
Chapter 5

PLUMA Example Full Pipeline and Configuration File

The PLUMA source release includes a folder of example configurations and selected test data. Examples are organized by purpose, and each folder contains one or more pipeline configuration files. Some configurations include configuration files for both the CPU and GPU, and others run the same algorithm (i.e. ATria) on multiple biological systems.

We take a closer look now at the full pipeline example that we used to describe the structure of the PLUMA configuration file:

```
# Metagenomics analysis pipeline
Prefix data/Stability
Plugin Mothur inputfile Stability.mothur outputfile none
Plugin CountTableProcessing inputfile stability.trim.abund.pick.an.unique_list outputfile input.Stability.csv
Plugin CSVNormalize inputfile input.Stability.csv outputfile input.Stability.normalized.csv
Plugin Correlation inputfile input.Stability.normalized.csv outputfile correlations.pvalued.csv
Plugin CSVPad inputfile correlations.pvalued.csv outputfile Stability.pvalued.csv
Plugin GPUATria inputfile Stability.pvalued.csv outputfile Stability.pvalued.ATria.noa
# Plugin ATria inputfile Stability.pvalued.csv outputfile Stability.pvalued.ATria.noa
Plugin CSV2GML inputfile Stability.pvalued.csv outputfile Stability.pvalued.gml
Plugin Cytoscape inputfile Stability.pvalued.gml outputfile none
```

This configuration file is a terrific example of the integration of many plugins with different implementation languages, some generated by the PluginGenerator, and others constructed from scratch. Figure 5.1 illustrates this. Mothur and Cytoscape are standalone software tools for which we produced C++ plugins using the PluginGenerator. We implemented three of the plugins (CSVNormalize, CSVPad, and CSV2GML) in Python, two (CountTableProcessing and Correlation) in R and one in CUDA (GPUATria).

We now outline the details of each stage of the pipeline. Some stages are simple file conversions, others are more involved - but the setup is the same with respect to the PLUMA configuration file.
5.1 Stage 1: Mothur

As mentioned, this plugin was automatically generated through the PluginGenerator module and calls the mothur executable assuming it is in your system PATH. The input file Stability.mothur runs the example from the Mealybugs tutorial [31] for Mothur using input data from Illumina’s MiSeq [50] platform. In this case, we start from raw sequence data and perform a series of steps to reduce errors in the reads, followed by a removal of chimeric sequences, and an assessment of error rates (steps 4-7 of the tutorial). This section will end by clustering these final sequences into Operational Taxonomic Unit (OTUs). Mothur does this using similarity-based clustering [63] to compute a distance between sequences. It then references a database (we use SILVA [47]) to take each cluster and map to the most specific phylogenetic classification of microbe.

Mothur produces a number of output files without having to specify them in the PLUMA configuration file. The two with which we are concerned are: (1) the abundance of each OTU (classified by a unique identifier) in each sample (*.shared, Program 9), and (2) the mapping of these same OTU identifiers to OTU classifications (*.cons.taxonomy, Program 10). These each start with the same file prefix, which we feed into the next stage of our pipeline.

<table>
<thead>
<tr>
<th>Program 9 File of OTU abundances produced by Mothur (*.shared file).</th>
</tr>
</thead>
<tbody>
<tr>
<td>be1</td>
</tr>
<tr>
<td>0.03</td>
</tr>
<tr>
<td>0.03</td>
</tr>
<tr>
<td>0.03</td>
</tr>
</tbody>
</table>

Figure 5.1: Conceptual description of our FullPipeline example. Plugins for Mothur and Cytoscape were generated automatically PLUMA’s PluginGenerator module, and the rest were written in one of several different languages.
Program 10 Mapping of OTUs to classifications produced by Mothur (.taxonomy file).

<table>
<thead>
<tr>
<th>OTU</th>
<th>Size</th>
<th>Taxonomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otu001</td>
<td>12283</td>
<td>Bacteria (100); ‘Bacteroidetes’ (100); ‘Bacteroidia’...</td>
</tr>
<tr>
<td>Otu002</td>
<td>8888</td>
<td>Bacteria (100); ‘Bacteroidetes’ (100); ‘Bacteroidia’...</td>
</tr>
<tr>
<td>Otu003</td>
<td>7789</td>
<td>Bacteria (100); ‘Bacteroidetes’ (100); ‘Bacteroidia’...</td>
</tr>
<tr>
<td>Otu004</td>
<td>7452</td>
<td>Bacteria (100); ‘Bacteroidetes’ (100); ‘Bacteroidia’...</td>
</tr>
<tr>
<td>Otu005</td>
<td>7425</td>
<td>Bacteria (100); ‘Bacteroidetes’ (100); ‘Bacteroidia’...</td>
</tr>
<tr>
<td>Otu006</td>
<td>6583</td>
<td>Bacteria (100); ‘Bacteroidetes’ (100); ‘Bacteroidia’...</td>
</tr>
<tr>
<td>Otu007</td>
<td>6323</td>
<td>Bacteria (100); ‘Bacteroidetes’ (100); ‘Bacteroidia’...</td>
</tr>
</tbody>
</table>

5.2 Stage 2: CountTableProcessing

The goal of this plugin (written in R) is to take OTU names and abundances in each sample from Mothur and produce an abundance matrix, with rows as samples and columns as OTUs, and $M_{i,j}$ corresponds to the abundance of OTU $j$ in sample $i$.

CountTableProcessing produces this output file in CSV format (Program 11), a format recognized by many spreadsheet applications including Microsoft Excel. This plugin can in a sense be viewed as a tool that merges Mothur’s output list of OTUs (which consists of identifiers, counts over all samples, and classifications at each level of the taxonomic ranking), and abundances for each sample (which consists of sample identifiers, OTU identifiers, and their abundances in a table). The CSV file produced by this plugin will consist of a table similar to the one in this latter file produced by Mothur, but uses OTU names instead of identifiers for columns. For an OTU name, the plugin takes the most specific classification level produced in the Mothur output for each identifier.

Program 11 OTU abundance matrix produced by Stage 2 of the pipeline.

```
‘...’, ‘Family. Porphyromonadaceae.0001’, ‘Family. Porphyromonadaceae...
‘F3D0’, 500, 307, 392, 404, 631, 352, 168, 163, 136, 19, 26, 113, 52, 296, 101, 78...
‘F3D1’, 350, 309, 187, 63, 73, 115, 131, 178, 84, 112, 53, 34, 116, 71, 247, 0, 280...
‘F3D141’, 387, 335, 297, 483, 426, 279, 205, 333, 117, 181, 111, 99, 9, 75, 15, 99...
‘F3D142’, 244, 258, 142, 151, 253, 191, 203, 81, 72, 45, 51, 82, 104, 10, 6, 478...
‘F3D143’, 189, 152, 174, 206, 310, 192, 117, 91, 60, 83, 68, 41, 37, 17, 2, 357...
‘F3D144’, 344, 243, 259, 316, 467, 280, 133, 36, 136, 269, 93, 85, 13, 29, 6, 105...
...
```

5.3 Stage 3: CSVNormalize

The third stage of this pipeline (written in Python) normalizes the abundance matrix produced by CountTableProcessing across columns, so that each sample’s OTU counts correspond to a portion of the total number of OTUs classified in that sample. The output of this plugin is also in CSV format, set up identically to its input file but using normalized instead of raw abundances as shown in Program 12.
### 5.4 Stage 4: Correlation

As a next step, we take these normalized abundances and produce a *correlation matrix*, which provides an estimate of how well pairs of OTU abundances *correlate* with each other. These correlations can be positive or negative. A strong positive correlation between OTU $i$ and $j$ would be indicated by a direct relationship between the abundances of $i$ and $j$ in each sample, i.e. if $i$ is higher in sample $A$ compared to sample $B$, then $j$ is also higher in sample $A$ compared to sample $B$. A strong negative correlation between OTU $i$ and $j$ would be indicated by an inverse relationship between their abundances. Zero correlation would indicate that there is no definite relationship between their abundances. Correlations can also be weakly positive and negative, based on the strength of their direct (inverse) relationships. The plugin computes correlation by looking at the values of $i$ and $j$ across all sets of samples.

Correlation is an R plugin and uses the `rcorr` method of the R `Hmisc` package, which produces a matrix of correlations and P-values. These values will be thresholded at a P-value of 0.01, which is a modifiable global variable as mentioned earlier.

After running `rcorr`, this plugin does the final processing to produce an output CSV file where this time OTU names occupy both rows and columns, and $M_{i,j}$ indicates the correlation between OTU $i$ and OTU $j$. Program 13 shows this structure. Note that this matrix is symmetric, i.e. $M_{i,j} = M_{j,i}$. It thus can be represented as a signed and weighted undirected network where OTUs are nodes and correlations are edges, as shown in Figure 5.2. We use this approach to perform downstream analysis in Stage 6.

---

**Program 12** OTU normalized abundance matrix produced by Stage 3 of the pipeline.

```
'Family. Porphyromonadaceae.0001', 'Family. Porphyromonadaceae.0002',
'Family. Porphyromonadaceae.0001', 0.0814730324263, 0.0500244419097, 0.0638748574222, 0.065830211,
'Family. Porphyromonadaceae.0001', 0.0760869565217, 0.0671739130435, 0.040652173913, 0.013695652,
'Family. Porphyromonadaceae.0001', 0.0835672640898, 0.072338587778, 0.0641330166271, 0.104297111,
'Family. Porphyromonadaceae.0001', 0.101328903654, 0.107142857143, 0.0589700996678, 0.062707641,
'Family. Porphyromonadaceae.0001', 0.0792120704107, 0.0637049455155, 0.0729253981559, 0.086336111,
`...
```

**Program 13** OTU correlation matrix produced by Stage 4 of the pipeline.

```
'Family. Porphyromonadaceae.0001', 'Family. Porphyromonadaceae.0002',
'Family. Porphyromonadaceae.0001', 0.820245563983917, 0.579995274543,
'Family. Porphyromonadaceae.0002', 0.820245563983917, 1, 0.67020320892,
'Family. Porphyromonadaceae.0003', 0.579995274543762, 0.67020320892,
'Barnesiella.0001', 0.779624223709106, 0.874419510364532,
'Family. Porphyromonadaceae.0004', 0.0, 0, 0.779624223709106, 1,
'Family. Porphyromonadaceae.0005', 0.562049210071564, 0.874419510364532,
`...
```
Figure 5.2: The equivalent signed and weighted network corresponding to the correlation matrix in Program 13.
5.5 Stage 5: CSVPad

This plugin (written in Python) is about as simple a file conversion plugin as can be constructed. When assembling this pipeline, we actually created this plugin after the current plugins for Stage 4 and Stage 6. While Stage 6 (GPUATria, discussed in further detail next) expects a correlation matrix for input, the format is slightly different than that produced by the `write.table` function of R. The latter function produces a table with row and column labels, but there is no entry for the label where rows and columns intersect. The job of CSVPad is simply to append an empty string in that spot, as shown in Program 14.

While a straightforward modification to Stage 4 or (even easier) Stage 6 could have fixed this problem, we felt this was a good demonstration of the extensibility of PLUMA. Since file readers can be added as plugins without loss of generality, no modifications to Stage 4 or Stage 6 were required after testing them. We could simply add a new plugin to “pad” the CSV file output by Stage 4 to be compatible with Stage 6. This problem is now removed for the analysis community, if this situation arises again now a user can simply download this CSVPad plugin and drop it into their pipeline. This is much easier than attempting to modify their tools for compatibility with an existing framework.

Program 14 Padded correlation matrix produced by Stage 5 of the pipeline.

```
```

5.6 Stage 6: GPUATria

Once we have the correlation matrix we can perform some downstream network analysis, since as mentioned there is no difference between a correlation matrix and a signed and weighted network. In this network, OTUs are nodes and correlations are edges. Recent work on the microbiome has analyzed these types of networks, which are referred to as bacterial co-occurrence networks [29] or also microbial social networks [1]. The concept of a social network naturally arose from the basic property of co-occurrence networks, which is a measure of how often two entities appear “in tandem”. Biologically this could have several meanings, one microbe may be producing a nutrient that is critical to the other’s survival in a positive correlation, or may be producing a toxin that kills off another in a negative correlation.

Viewing these networks from the “social network” perspective, coupled with the fact that our sample co-occurrence networks upheld many properties of social networks (such as stability [17]), led to the application of social network algorithms to these networks at later pipeline stages. Ablatio Triadum (ATria) explores the social networking concept of centrality (or importance), in these networks. In summary, we would like to produce an ordered list of the most important bacteria in the network. Compared to other social network centrality algorithms, ATria does the best job at finding central nodes in different parts of the network. The three types of nodes it tends to find are: leader nodes of strongly connected components, villain nodes
(common enemies), and bridge nodes between connected components. To improve speed on large networks, this pipeline includes the version of ATria for the GPU (written in CUDA). The output of this plugin is an NOA file, which can be easily imported into Cytoscape. The NOA file consists of a simple table mapping node names to centrality values and ranks, as shown in Program 15.

**Program 15** NOA file of nodes and centrality values produced by Stage 6.

<table>
<thead>
<tr>
<th>Name</th>
<th>Centrality</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family. Ruminococcaceae.0009</td>
<td>47.1695</td>
<td>243</td>
</tr>
<tr>
<td>Oscillo bacter.0002</td>
<td>45.5364</td>
<td>242</td>
</tr>
<tr>
<td>Phylum. Firmicutes.0003</td>
<td>41.8464</td>
<td>241</td>
</tr>
<tr>
<td>Phylum. Firmicutes.0002</td>
<td>37.3408</td>
<td>240</td>
</tr>
<tr>
<td>Family. Lachnospiraceae.0004</td>
<td>33.3711</td>
<td>239</td>
</tr>
<tr>
<td>Phylum. Firmicutes.0013</td>
<td>28.8272</td>
<td>238</td>
</tr>
<tr>
<td>Order. Clostridiales.0017</td>
<td>25.9752</td>
<td>237</td>
</tr>
<tr>
<td>...</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.7 **Stage 7: CSV2GML**

In addition to needing this table, Cytoscape (Stage 8) will also need the network itself, but cannot visualize networks in CSV format. GML is an accepted format by Cytoscape, which is produced by this plugin. CSV2GML is thus a simple file converter plugin written in Python that converts an input CSV file into GML format, and its sample output is shown in Program 16. Upon completion of stages 6 and 7, we finally are ready to visualize the network in Cytoscape.

5.8 **Stage 8: Cytoscape**

The final pipeline stage, Cytoscape, was also produced by our PluginGenerator module. This plugin is responsible for visualizing the network produced in Stage 7 using Cytoscape. For this plugin to successfully run, Cytoscape must be in your system PATH. The Cytoscape window will automatically open and the network will be displayed, as shown in Figure 5.3. From there, you can also import the table produced by Stage 6 to give each node a “Centrality” attribute. This can in turn be used to color nodes, as shown in Figure 5.4.
Program 16 Equivalent GML version of the CSV file for the correlation network, in Program 14.

graph [
node [
id 0
label "Family.Porphyromonadaceae.0001"
]
node [
id 1
label "Family.Porphyromonadaceae.0002"
]
edge [
source 0
target 1
weight 0.820245563984
]
edge [
source 0
target 2
weight 0.579995274544
]
...

Figure 5.3: Co-occurrence network visualized using Cytoscape, opened in stage 8 of our pipeline.
Figure 5.4: Stage 8 co-occurrence network, colored by centrality value.
Chapter 6

The Future of PLUMA

Work is already underway to further expand PLUMA. We immediately plan to expand the Wrapper component of the software to include SWIG [6], which will allow compiled plugins to be accessible from scripted ones. This will facilitate compatibility across various programming languages, improving the ability to develop new ideas and reuse necessary existing components without additional overhead.

In addition we have developed an online plugin pool, and in the future would like members of the PLUMA user base will be able to deposit their plugins for other members to download and use in their pipelines. Achieving this will essential to our central purpose of PLUMA: we desire this to serve as a central portal for software pipelines, particularly those for various -omics analyses [55]. Additionally, the next release of PLUMA will hopefully include an easy plug-and-play graphical interface as a part of its User Layer, further improving ease of use. The program is currently being ported to Windows and will be released in the near future.
Appendix A

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Version 3, 29 June 2007

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### A.2 Contact Information

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