Shannon Human Splicing Pipeline Installation Guide and Documentation

Installation Guide:

Pre-installation requirements: Either CLC Genomics Workbench or CLC Genomics Workbench and CLC Genomics server, Perl and gcc*.

NOTE: If this installation occurs on a computer running MacOSX (Mountain Lion/Lion), gcc must be pre-installed. It is part of Apple's Xcode Developer package or can be obtained as a standalone installation from github (https://github.com/kennethreitz/osx-gcc-installer). Most standard Linux installations include gcc.

Standalone CLC Bio Genomics Workbench Version (Server license or support either undesired or unavailable)

1. Open CLC-Bio Workbench and select the "Plug-ins" button from the toolbar
2. Uninstall any previous version(s) of the Shannon pipeline for human splicing
3. Click 'install from file'.
4. Select "**CytognomixShannonPipeline.cpa**" software and click install
5. The Dependency plug-ins contain genomic annotations (Ensembl gene and dbSNP, hg reference sequence, Ri values of known splice sites) must be installed for use by the main pipeline on the Genomics Server. Like the software, these plug-ins are available at the CLC Bio website as part of your purchase. There are separate 'dependencies' plug-ins for each genome build available. Currently, dependencies plug-ins are available for hg18/NCBI36) and hg19/GRCh37).

    - If you intend to examine variants that are mapped to hg19 coordinates, click 'install from file', browse computer and select "**CytognomixDependenciesHG19**" (1.35 Gb) and click install.
    - If you intend to examine variants that are mapped to hg18 coordinates, the file "**CytognomixDependenciesHG18**" (1 Gb) must be installed in the same manner.
    - If variant data sources are mixed, ie. from both hg18 and hg19, both "**CytognomixDependenciesHG18**" and "**CytognomixDependenciesHG19**" must be installed.

- Keep in mind that at least one dependencies file must be installed for the pipeline to function. Otherwise an error is generated at run time.

6. Restart Genomics Workbench to complete installation**Installation of CLC Bio Genomics Server Version (Plugin will be run on the server only)**

1. First, install the CLC Bio Workbench version - as outlined above - under the heading "CLC Bio Genomics Workbench Version" but select "**CytognomixShannonPipelineClient.cpa**" instead of "CytognomixShannonPipeline.cpa". This version is unlicensed and will not run the Shannon pipeline without installation of the Genomics server plug-in.
2. Ensure the CLC-Bio Genomics Server is running. For more instructions on how to set up and access the server, see CLC-Bio's Genomics server documentation
3. Access the server through your web-browser and log in to the Server
4. Select the Plug-ins option in the Admin tab
5. Uninstall any previous version(s) of the Shannon pipeline for human splicing mutations plug-in from the server.
6. The Dependency plug-ins contain genomic annotations (Ensembl gene and dbSNP, hg reference sequence, Ri values of known splice sites) must be installed for use by the main pipeline on the Genomics Server. Like the software, these plug-ins are available at the CLC Bio website as part of your purchase. There are separate 'dependencies' plug-ins for each genome build. Currently, dependencies plug-ins are available for hg18/NCBI36) and hg19/GRCh37).

    - The dependencies plug-ins are large (≥1 Gb each). By default, the CLC-Bio server does not currently allow plug-ins of this size to be installed. To change the settings, under the Main configuration tab->HTTP settings: modify the 'Max upload size (MB)' value to ≥ 3000 Mb.
    - If you intend to examine variants that are mapped to hg19 coordinates, browse computer and select " **CytognomixDependenciesHG19**" (1.35 Gb) and click 'Install Plug-in'.
    - If you intend to examine variants that are mapped to hg18 coordinates, the file "**CytognomixDependenciesHG18**" (1 Gb) must be installed in the same manner.
    - If variant data sources are mixed, ie. from both hg18 and hg19, both "**CytognomixDependenciesHG18**" and "**CytognomixDependenciesHG19**" must be installed.
    - Keep in mind that at least one dependencies file must be installed for the pipeline to function. Otherwise an error is generated at run time.
7. The main Server pipeline plug-in can now be installed. Browse computer to select "**CytognomixShannonPipelineServer.cpa**" and click 'Install Plug-in'.

8. If you have not already set any 'File system locations' for your server as outlined in CLC-Bio's server documentation, this must be done before the Shannon pipeline can be run. The Server has to have a CLC Bio folder on your hard drive where CLC objects and files are defined in advance of running the plug-in. Please refer to the CLC Bio Genomics Server installation guide for details on Server set up.

Installation of Genomics Workbench Client-Genomics Server Version (both CLC Workbench and Server licenses are active)

1. First, install the CLC Bio Workbench version under the heading "Standalone CLC Bio Genomics Workbench Version (ie. " **CytognomixShannonPipeline.cpa**"). It is not necessary to install "CytognomixDependenciesHG18" or "CytognomixDependenciesHG19" if you never intend to use the Workbench computer to run the pipeline. (ie: every run will take place on the server). If you want to run the Shannon pipeline computations on both the Genomics Workbench and the Genomics Server, then the Dependencies plugins that you intend to use must to be installed on each of the respective computers. At least one "Dependency" plug-in must be installed on the Genomics Workbench when calculations are performed locally (without running the software on the Server).
2. Ensure the CLC-Bio Genomic Server is running. For more instructions on how to set up and access the server, see CLC-Bio's Genomics server documentation
3. Access the server through your web-browser and log in to the Server
4. Select the Plug-ins option in the Admin tab
5. Uninstall any previous version(s) of the Shannon pipeline for human splicing mutations plug-in from the Server.
6. The Dependency plug-ins contain genomic annotations (Ensembl gene and dbSNP, hg reference sequence, Ri values of known splice sites) must be installed for use by the main pipeline on the Genomics Server. Like the software, these plug-ins are available at the CLC Bio website as part of your purchase. There are separate 'dependencies' plug-ins for each genome build. Currently, dependencies plug-ins are available for hg18/NCBI36) and hg19/GRCh37).

    - The dependencies plug-ins are large (≥1 Gb each). By default, the CLC-Bio server does not allow plug-ins of this size to be installed. To change the settings, under the Main configuration tab->HTTP settings: modify the 'Max upload size (MB)' value to ≥ 3000 Mb.
    - If you intend to examine variants that are mapped to hg19 coordinates, browse computer and select " **CytognomixDependenciesHG19**" (1.35 Gb) and click 'Install Plug-in'.

- If you intend to examine variants that are mapped to hg18 coordinates, the file "**CytognomixDependenciesHG18**" (1 Gb) must be installed in the same manner.
- If variant data sources are mixed, ie. from both hg18 and hg19, both "**CytognomixDependenciesHG18**" and "**CytognomixDependenciesHG19**" must be installed.
- Keep in mind that at least one dependencies file must be installed for the pipeline to function. Otherwise an error is generated at run time.

7. The main pipeline plug-in can now be installed. Browse computer to select "**CytognomixShannonPipelineServer.cpa**" and click 'Install Plug-in'.
8. If you have not already set any 'File system locations' for your server as outlined in CLC-Bio's server documentation, this must be done before the Shannon pipeline can be run. The Server has to have a CLC Bio folder on your hard drive where CLC objects and files are defined in advance of running the plug-in. Please refer to the CLC Bio Genomics Server installation guide for details on Server set up.

* Requirements and validation

The Cytognomix Shannon human mRNA splicing plug-in runs in standalone mode on the CLC Genomics Workbench V5.5 or with both the Workbench and CLC Genomics Server V.4.5 (as a standalone server or running Gridworks). Released for **Linux** and **MacOSX** Operating systems supporting Perl and gcc. Installation has been verified with Perl v.5.8.8 and 5.10.1 and gcc v.4.1.2 and v.4.4.3 with the Ubuntu 2.6.32-27 (32 and 64 bit), CentOS 2.6.18-238 (64 bit), and Fedora 16 (32 bit) kernels, and MacOSX (Mountain Lion release version 10.8, Lion release version 10.7.4; gcc v.4.2.1 and Perl 5.12.3 and 5.12.4) on hardware equipped with an Intel I7 processor and at least 4Gb RAM.

Quick Start

This page contains information on how to run the plugin. For an overview of information theory please view "Review"
Note: This guide assumes that the plug-in has been installed. For help installing the Shannon Human Splicing Pipeline, please consult the installation guide.

Importing Data
Before any analysis can take place, input data containing variants is needed. The data to be examined must be in one of two formats. In either format, the pipeline does not currently accept indels.

Option 1:
chromosome[tab]unique_identifier[tab]coordinate[tab]reference/variant
For example the line:
3 8 90443689 G/A
is acceptable provided the variant in question is located on chromosome 3 at coordinates 90443689 and modifies the reference G to an A.

To import this type of data, click the import button on the taskbar in the CLC-Bio workbench, select the file to be imported, and **select force import as type: Shannon Pipeline Basic Format** (see Figure 1 below).

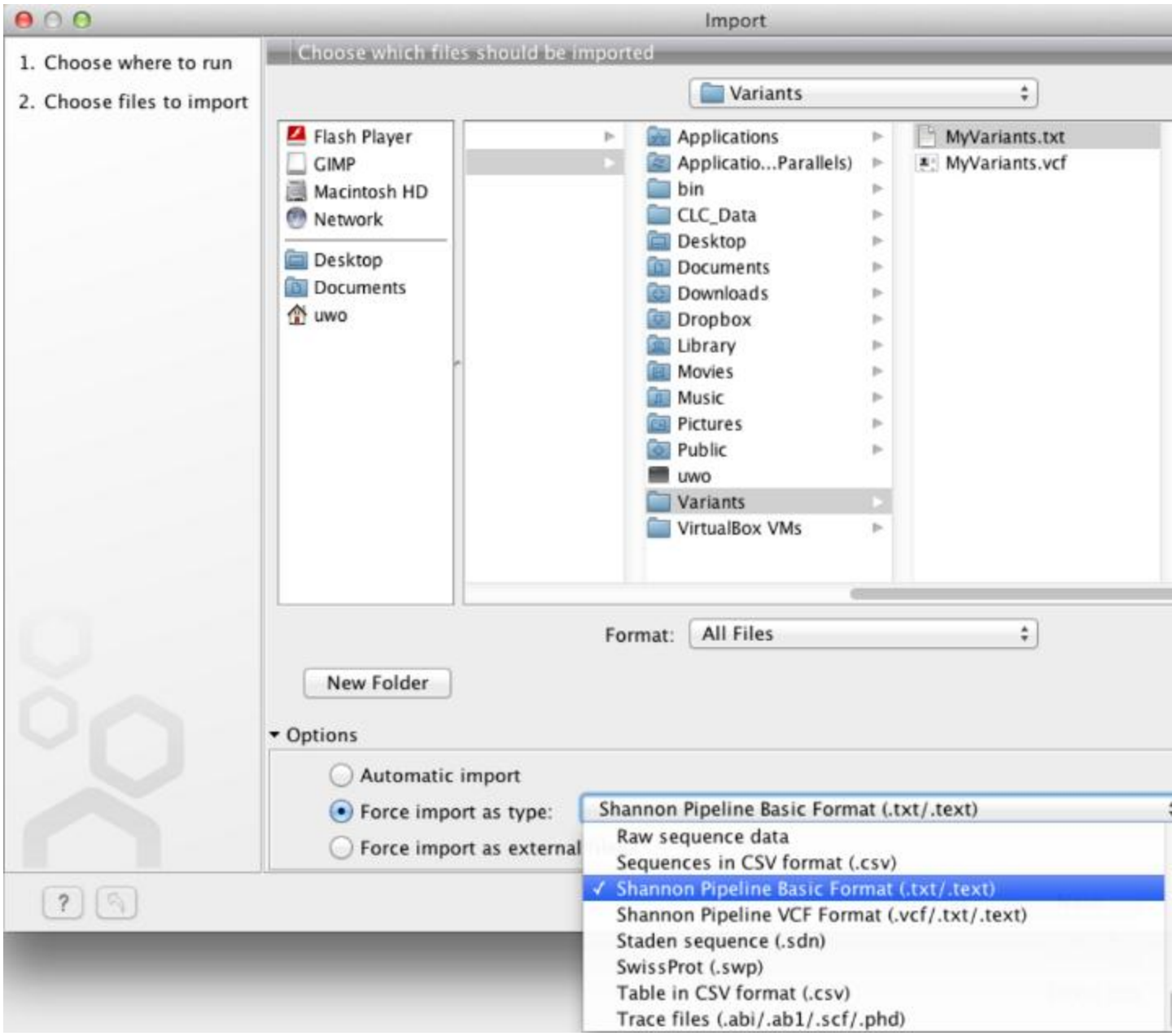

Figure 1. A demonstration of the Option 1 (Basic) import process. Select force import as: Shannon Pipeline Basic Format

Option 2:
VCF files may be imported. The file must be a standard VCF file with at least the first five columns present. The necessary fields are CHROM, POS, ID, REF, ALT in that order. File headers are not necessary and will be ignored if present.
For example, the following lines are acceptable:
5 148835675 . C T
5 148989410 ID1 A G,T

Please keep in mind that although VCF format can be imported, the Shannon Pipeline cannot accept indels. There must not be indels present in VCF files to be imported. **Indels present will be ignored without warning**

To import the data, click the import button on the taskbar in the CLC-Bio workbench, select the file to be imported, and **select force import as type: Shannon Pipeline VCF Format**.

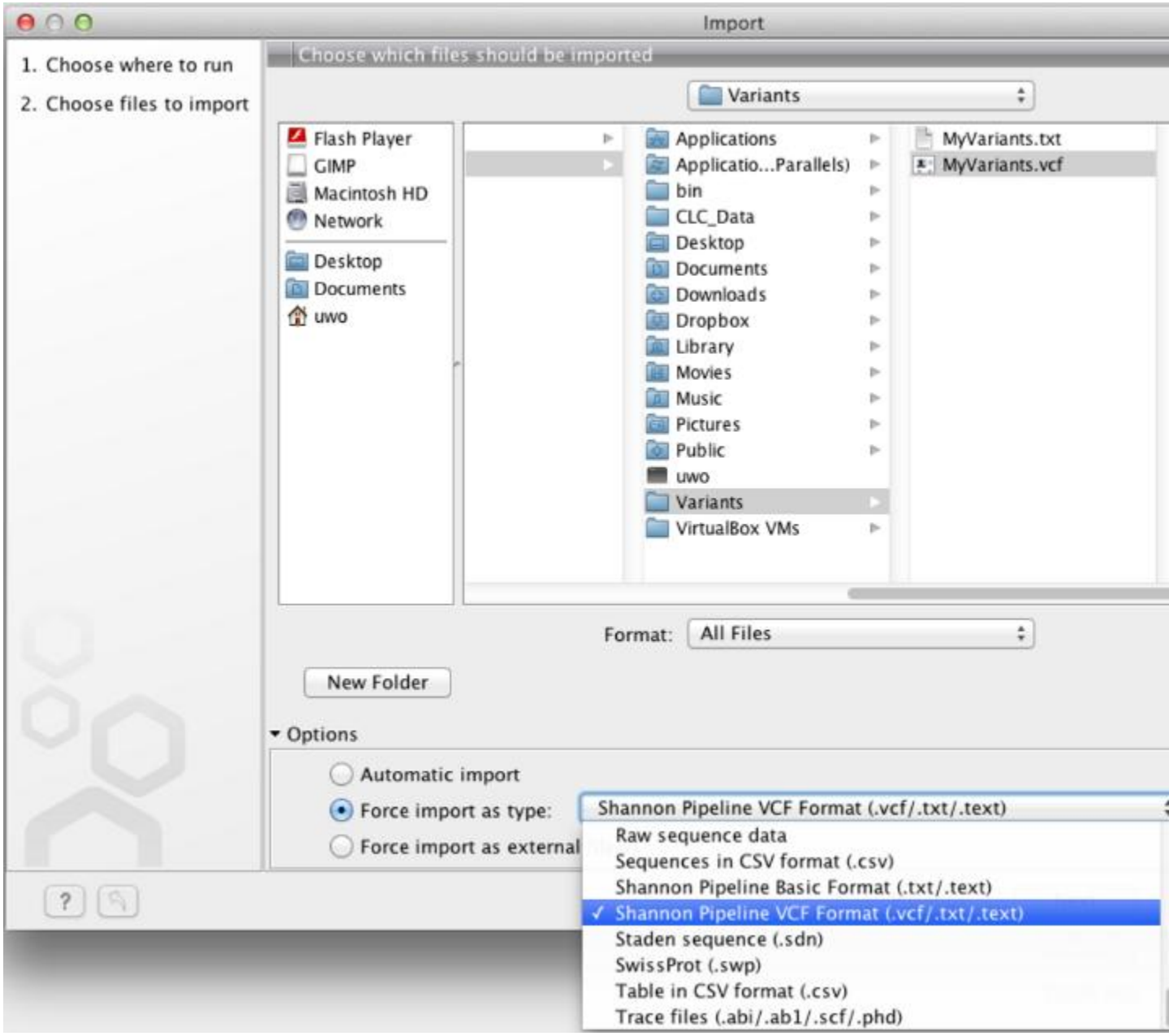

Figure 2. A demonstration of the Option 2 (VCF) import process. Select force import as: Shannon Pipeline VCF Format

Example Data
Example data was automatically placed in the directory "ExampleData_ShannonPipeline" upon plugin installation. Three descriptively named objects can be found in the directory:

- 1. Pre-Imported-hg19-Variants_Ready-to-be-examined-by-the-Shannon-Pipeline: The result of either importing file 2 using Option 1 in the import section above or importing file 3 using Option 2. This object may be used to test the Shannon Pipeline.
- 2. SampleBasicFormat_Would-be-imported-by-forcing-import-as-Shannon-Pipeline-Basic-Format.txt: This is an example of the Shannon Pipeline Basic format before importing.
- 3. SampleVCF_Would-be-imported-by-forcing-import-as-Shannon-Pipeline-VCF.vcf: An example of the Shannon Pipeline VCF format before importing

This example data was included to act as a starting point for first time users of the plugin. If it is no longer needed, the ExampleData_ShannonPipeline may be deleted. If the ExampleData_ShannonPipeline folder was accidentally deleted, please reinstall the plugin as the example files are placed in the directory upon installation.

Running the pipeline
After importing your data and clicking on "Launch Shannon Pipeline" (located in the toolbox, under Shannon Human Splicing Pipeline), a wizard will appear asking whether you would like the analysis to take place on the server or the workbench. Each step of this wizard is described below:

- Step 1: Select the desired analysis location (workbench, CLC server, or grid)
- Step 2: Select your imported variant data (Data **must** be imported using one of the two methods above. See the importing and example data sections above for help. The file Pre-Imported-hg19-Variants_Ready-to-be-examined-by-the-Shannon-Pipeline in the ExampleData_ShannonPipeline can be used in this step if you have not imported your own data yet.)
- Step 3: If desired, options to filter the data generated by the pipeline can be selected here. Note that if a variant is filtered out by these options it cannot be viewed unless the pipeline is run again with relaxed filters. It is generally advisable to leave the filters as relaxed as possible (default) and sort/filter if necessary after the results are generated
- Step 4: Select whether you would like to save or open your results. We suggest that you select save and check "make log".
- Step 5: Select your desired location for the results data
- Upon clicking finish, the pipeline will begin analysis
- Only one analysis can be run at a time

A pop-up window indicates the run is "Done" when complete.
Your results are located in the directory specified in the final step of the Launch Shannon Pipeline wizard. They include: tabular output split into 4 files (complete, inactivating, leaky, and cryptic), plots for every chromosome and a genome-wide Manhattan style plot which includes all the variants. The "Command Line Output" is available for those interested in seeing the console output of the pipeline.

Displaying results

In the navigation area of the workbench, double click a tabular or plot results object. The objects will be displayed in an appropriate editor.

Tables

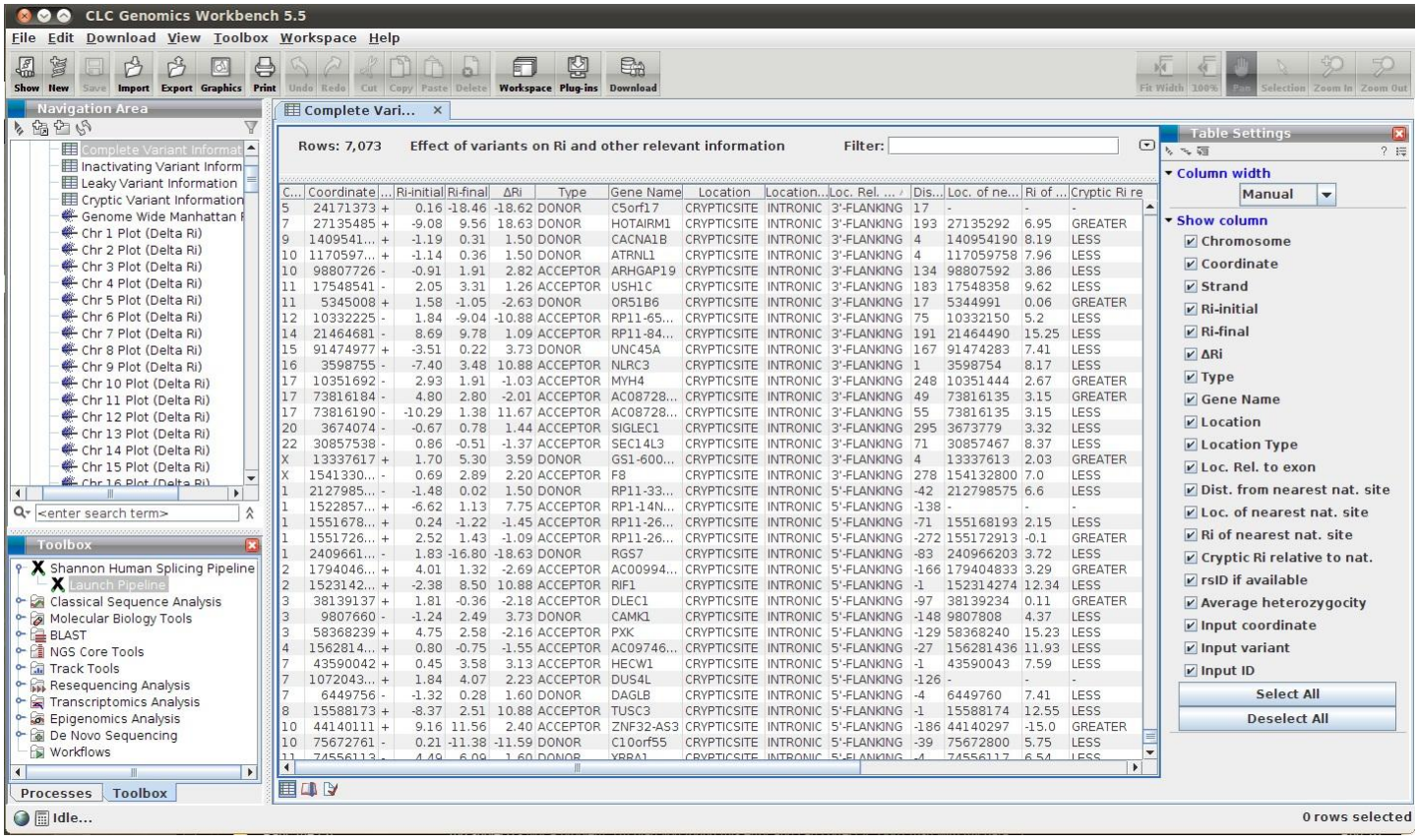

Figure 1. An example of a table generated by the pipeline. Columns which are not of immediate interest to you can be temporarily removed by unchecking the checkboxes within the sidebar on the right.

The tables contain all information gained through the information analysis on variants. Four tables are generated each time the pipeline is executed, these are:

1. Complete Variant Information

- All sites exhibiting a delta R_i of at least 0.1 are included here

2. Inactivating Variant Information

- Includes natural site variants with an original R_i greater than 1.6 and which drop below that value after the variant is introduced

3. Leaky Variant Information

- Natural site variants which experience a drop in R_i after the variant is introduced

4. Cryptic Variant Information

- Includes only cryptic site variants

Each row of the table represents a single variant. The meaning of each column is described below:

1. Chromosome
2. Coordinate
3. Strand

- Displayed as "+" for positive and "-" for negative strand

4. R_i-initial

- Ri of the site before introducing the variant

5. R_i-final

- Ri of the site after introducing the variant

6. Delta R_i

- The change in R_i before and after introducing the variant

7. Type

- The site is either an acceptor or a donor. Displayed as "ACCEPTOR" or "DONOR"

8. Gene Name

- Name of the gene closest to the location of the variant

9. Location

- The site is either natural or cryptic. Displayed as "NATURALSITE" or "CRYPTICSITE"

These columns are included only for cryptic site variants

10. Location Type

- If the location of the variant is within an exon it is "EXONIC". Otherwise, it is "INTRONIC"

11. Location relative to exon

- If the location of the variant is "INTRONIC" and within 300 base pairs of an exon, depending on its location relative to the exon it is "3'-FLANKING" or "5'-FLANKING"

12. Distance from nearest natural site

- If the location of the variant is within 1000 base pairs of a natural site, the number of base pairs separating the two is shown here

13. Location of nearest natural site

- If the location of the variant is within 1000 base pairs of a natural site, the coordinates of the nearest natural site are shown here

14. R_i of nearest natural site
15. Cryptic R_i relative to natural site R_i

- If a cryptic site has a higher R_i than the nearest natural site after the variant is introduced it is "GREATER", otherwise it is "LESS"

16. rsID if available

- dbSNP130 is examined to determine if the variant in question is a known variant. If it is found within dbSNP130, its rsID is displayed

17. Average heterozygosity

- Similarly, if the variant is found in dbSNP130, its average heterozygosity is displayed

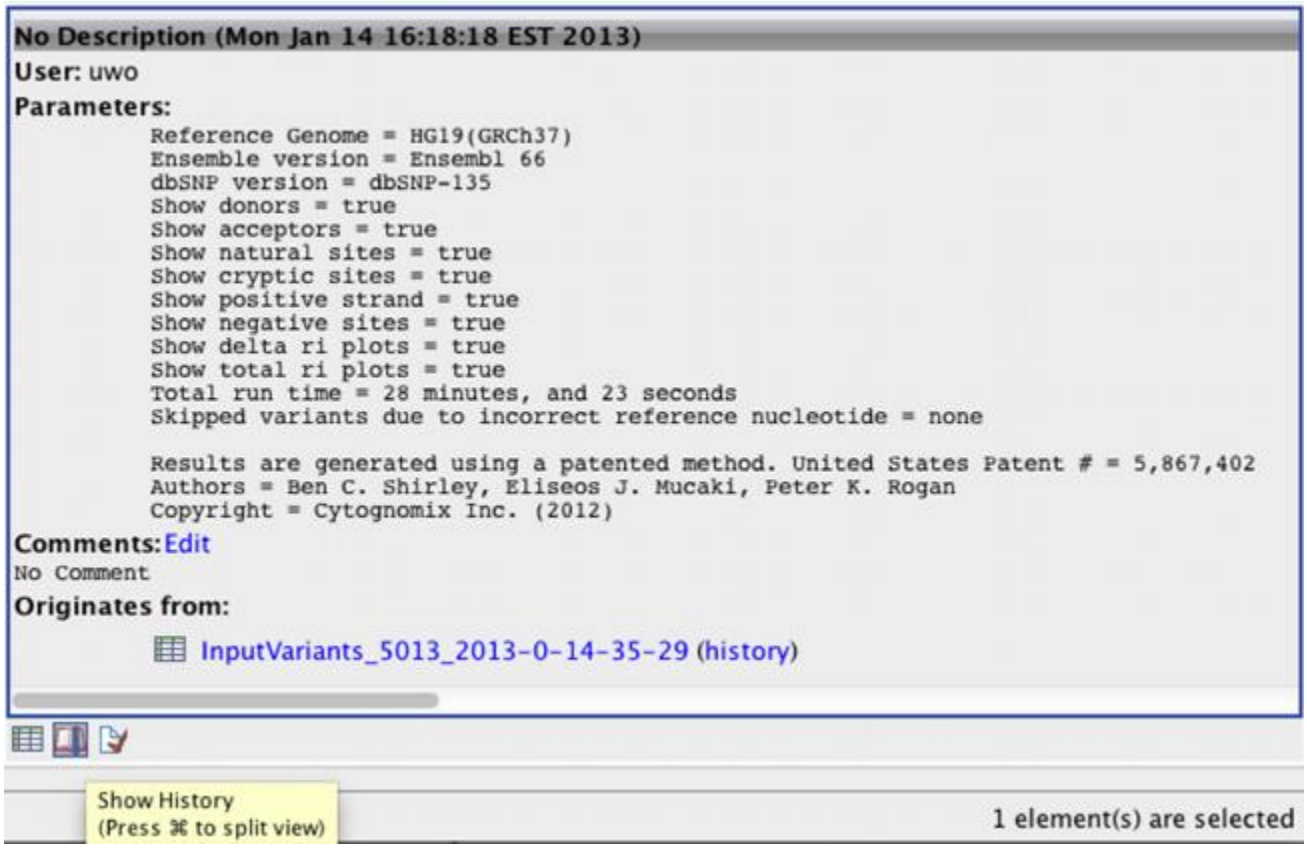

Figure 2**.** An example of table history generated by the pipeline. While the table editor is open (such as in figure 1), select history at the bottom of the screen. Genome version, Ensembl version, filer options, run time, skipped variants, and the input file for the appropriate pipeline execution can be found here.

Plots

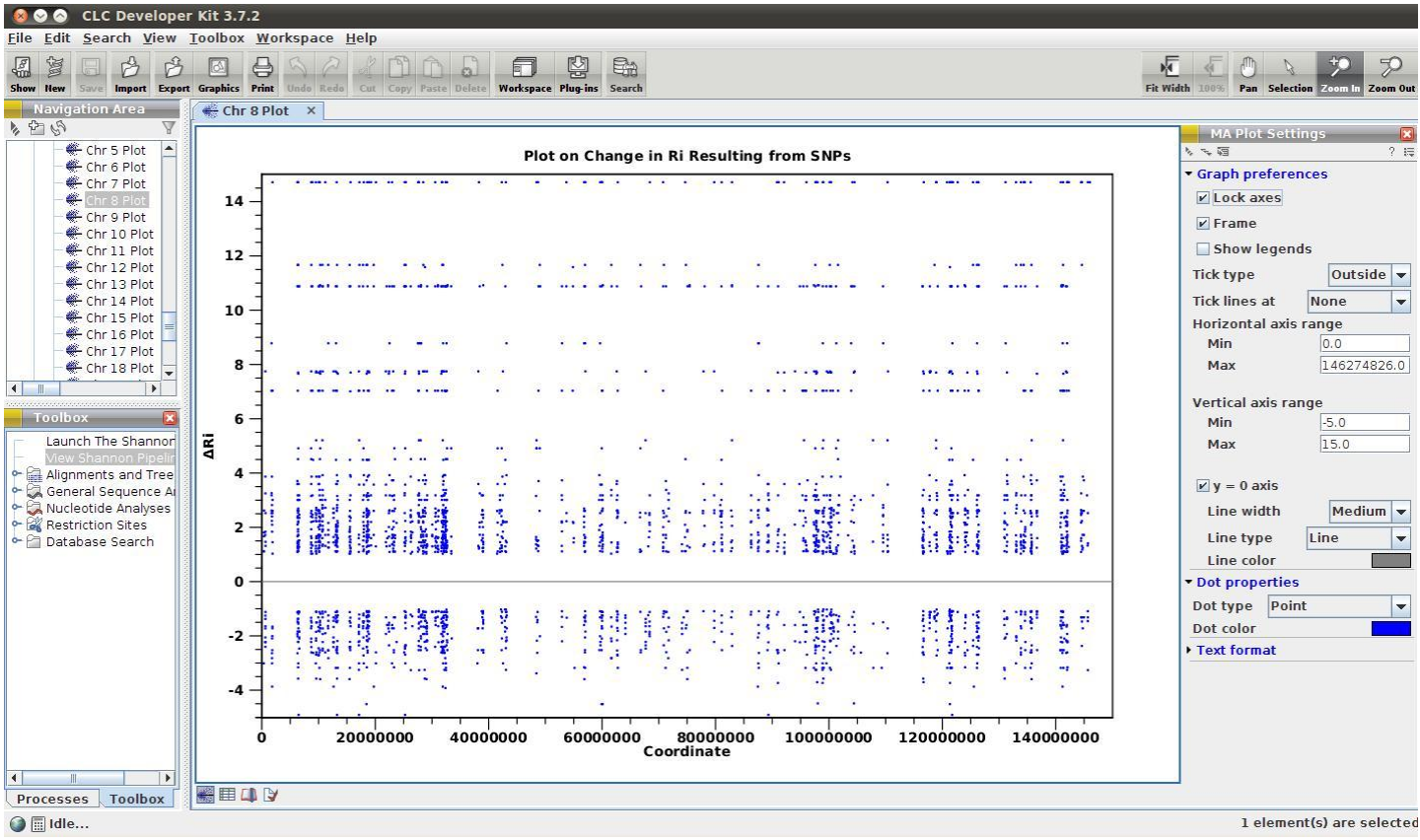

Figure 1. An example of a plot generated by the pipeline.

The plots provide a visual representation of the delta Ri for each variant. The genome wide, Manhattan style plot shows variants across the whole genome. Plots for individual chromosomes provide a closer look at the effect of the variants on a single chromosome.

If a variant on the plot is of interest to you, hovering over its plot point will produce a tool-tip containing information about the variant:

- Chromosome
- Coordinate
- Delta Ri (change in Ri before and after variant is introduced)
- Final Ri (Ri after variant is introduced)
- rsID from dbSNP130/135 if available

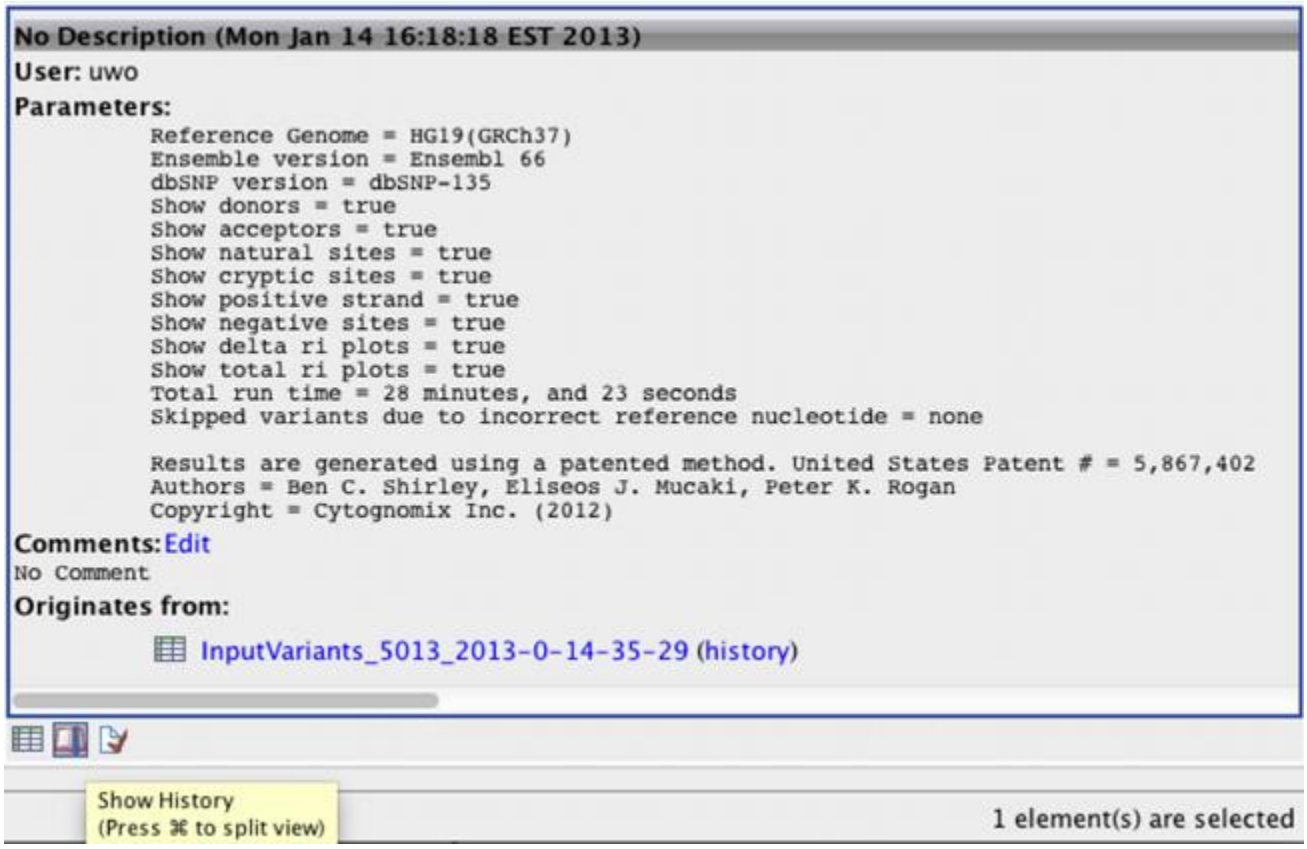

Figure 2. An example of plot history generated by the pipeline. While the plot editor is open (such as in figure 1), select history at the bottom of the screen. Genome version, Ensembl version, filer options, run time, skipped variants, and the input file for the appropriate pipeline execution can be found here.

Tracks

Four BED graph tracks are generated each time the pipeline is executed, these are:

1. customtrack-positive-acceptor-deltaRi (acceptor sites on the positive strand)
2. customtrack-negative-acceptor-deltaRi (acceptor sites on the negative strand)
3. customtrack-positive-donor-deltaRi (donor sites on the positive strand)
4. customtrack-negative-donor-deltaRi (donor sites on the negative strand)

Each row of a track represents a single variant. Each track has a header automatically included and are ready to be viewed using a genome browser. The header hides other tracks and displays ensGene (Ensembl Gene Predictions).

Example row:
chr1 8863452 8863452 14.7147693634033
On chromosome 1, coordinate 8863452 the predicted result of the input variant is an Ri increase of 14.71 bits. When viewed in a genome browser, a vertical line depicts the change in Ri.

FAQ

This FAQ will be updated with answers to common questions.

Q: Does the Shannon pipeline handle indels?
A: Not at the present time. However, future versions of the plugin will.

Q: How far away from an exon does the pipeline look for cryptic splice sites?
A: Currently, the pipeline looks up to 100bp from the exon boundary and within exons.

Q: How fast is the Shannon Human Splicing Pipeline?
A: In our testing, the pipeline averaged 3343 variants/min on an I7-based server.
100,000 variants took 37min to analyze. Incresing the number of variants leads to an approximately linear increase in computation time (314,637 variants in 87min).

Q: If something has gone wrong, how can I find out more about the problem?
A: An object ServerStdErrLog.log will appear with the output objects if an error was enountered during the run

Q: If my variant is on the complementary strand, will the Shannon Human Splicing Pipeline process it?
A: Yes, the variant is complemented to match the substitution on the reference sequence.

Q: My VCF file is not importing properly using force import as Shannon Pipeline VCF Format. What can I do?
A: We have been made aware that some special characters rarely found in VCF files can cause the import to fail. Generally, those characters are not found in the columns required by the pipeline (the first 5 columns). Please try creating a copy of your VCF file and remove all extraneous columns before importing again.

Shannon Human Splicing Pipeline

References

These are references for information theory based splice site analysis.

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Shannon Human Splicing Pipeline

Requirements

Installation tested on the following system setups
Perl: 5.8.8, 5.10.1, 5.12.3, 5.14.2. gcc: 4.1.2, 4.2.1, 4.4.3, 4.6.3, . kernel: Ubuntu 2.6.32, CentOS 2.6.18, Fedora 3.1.0-7, Mac OSX (Lion) 10.7.4.

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