

iCellR Plugin

Introduction

The iCellR plugin is built on the R package ‘iCellR’ by Alireza “Reza” Khodadadi-Jamayran, a Senior Bioinformatics Software Engineer at NYU. The iCellR tool is designed to analyze high parameter single-cell data in R. The iCellR plugin by BD Life Science - Informatics extends this functionality to users who work with data from scRNA-seq data in SeqGeq, or even flow cytometry data in FlowJo. Users can perform: clustering (from the nbClust R package), tSNE, UMAP, and PCA analyses - simultaneously - and view the results in an interactive 3D plot using GoogleChrome. In SeqGeq, there is additional functionality to perform Differential Expression Analysis on a categorical clustering parameter based on an unsupervised clustering algorithm, or SampleID, at the click of a button. Available for both FlowJo and SeqGeq, with some dependencies in R.

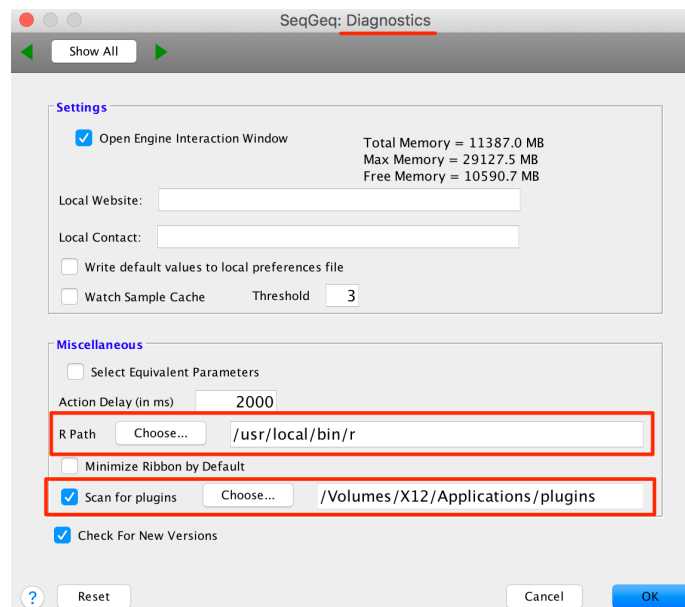
Please review FlowJo documentation for installing plugins <http://docs.flowjo.com/d2/plugins/installing-plugins/>.

Video Illustrations of the DankPipeline Functionality

To see the plugin in action (within SeqGeq), check out the quick tutorial here: <https://tinyurl.com/dankrootcellr-demo-v0-2>

Download and Installation

1. Place the plugin .jar file in your Plugins folder, and direct FlowJo to that folder using the Diagnostics section of the Preferences.
2. Make sure you have R installed and the R path is specified in the R Path field of the Diagnostics section of the Preferences.



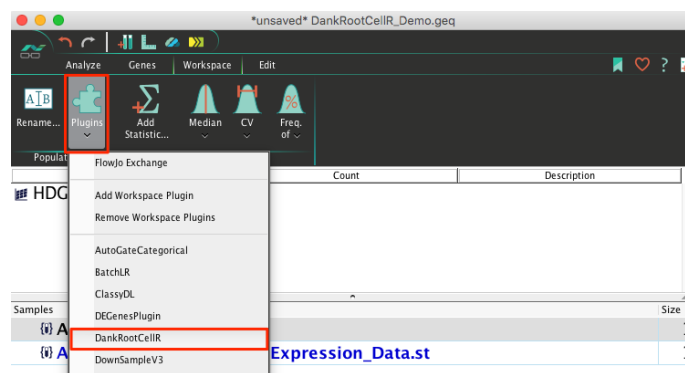
3. Place the .jar files into the Plugins folder.
4. Restart the SeqGeq or FlowJo application to pick up the new plugin. When the plugin is started for the first time, it should take a bit longer to calculate - This is because the plugin should automatically try to install the required R packages. The iCellR plugin was tested with iCellR version 1.1.2, and plotly version 4.9.0. If the auto-install fails, the iCellR R dependencies can be manually installed by typing (or copy/pasting) the following in your R console:

```
install.packages(c("iCellR", "plotly", "ggplot2", "Matrix"))
```

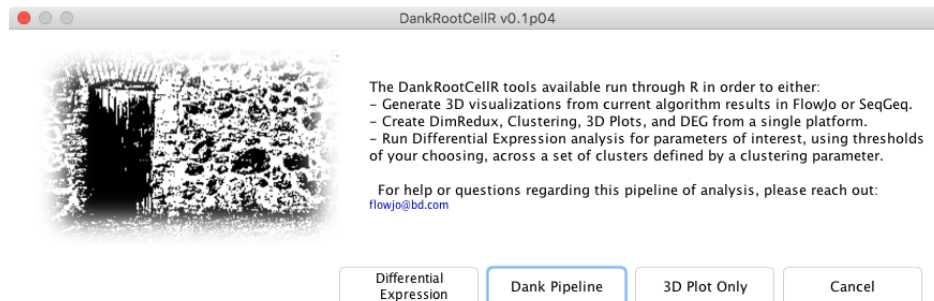
Usage

To run the iCellR plugin on your file,

1. Select the population of interest within the workspace.
2. Go to the Workspace tab and select the **iCellR** option from within the Plugins dropdown there. Note that plugins will be unavailable (greyed out) if no population is selected.

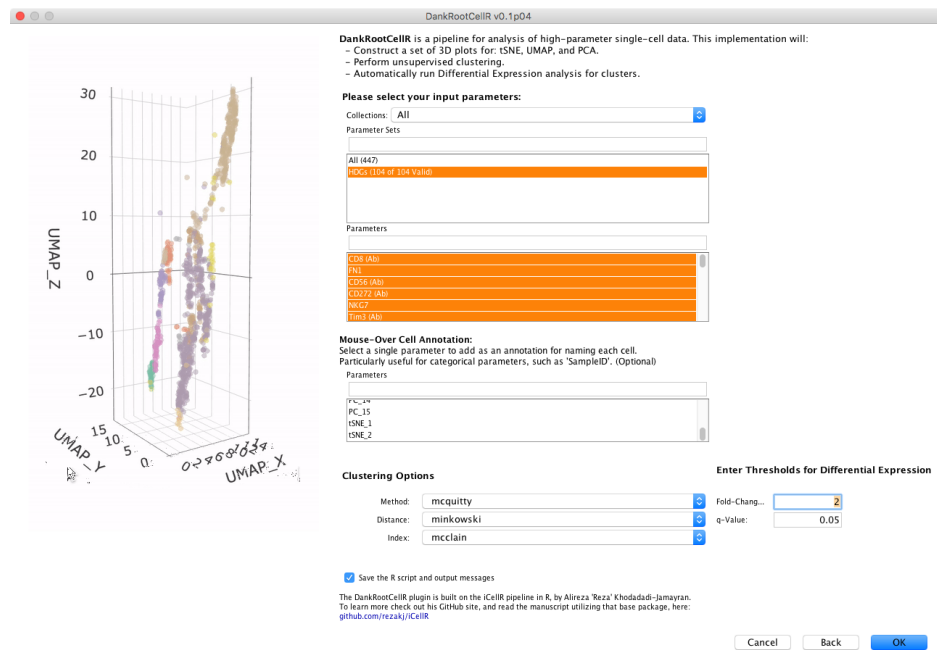


3. This will bring up a dialog where researchers can choose which tool to begin with. The options include:
 1. Running **Differential Expression** analysis for parameters of interest across a set of clusters defined by a clustering parameter. (*SeqGeq Only*)
 2. **iCellR Pipeline** to create DimRedux (PCA + tSNE + UMAP), Clustering, 3D plots, and DEG.
 3. **3D Plots** to generate 3D visualizations from any parameters available in FlowJo or SeqGeq.

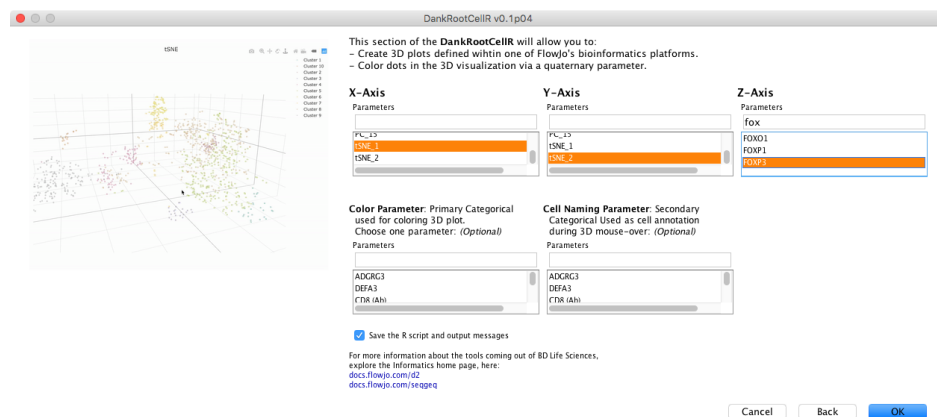


- iCellR Pipeline** When starting the iCellR Pipeline, you will need to select a parameter set of interest, then either select all of those parameters to use for PCA, tSNE, and UMAP or a subset. Optionally, one can choose a categorical parameter, such as ‘SampleID’ to use for Mouse-over annotations. Next, choose clustering options and thresholds for Differential Expression analysis. Once completed, you will see new Gene Set Collections (Up and Down Regulated) displayed in the Gene Sets pane of the SeqGeq workspace. A web browser will also open up and display interactive 3D plots of principal components, tSNE, and UMAP. These interactive plots can also be exported as static png images from within the browser. (*pro-tip: use a screen-recording utility such as Quick-Time to record the interactive session with the 3D figure of interest, and convert that to a video gif, for a mind blowing analysis review*)

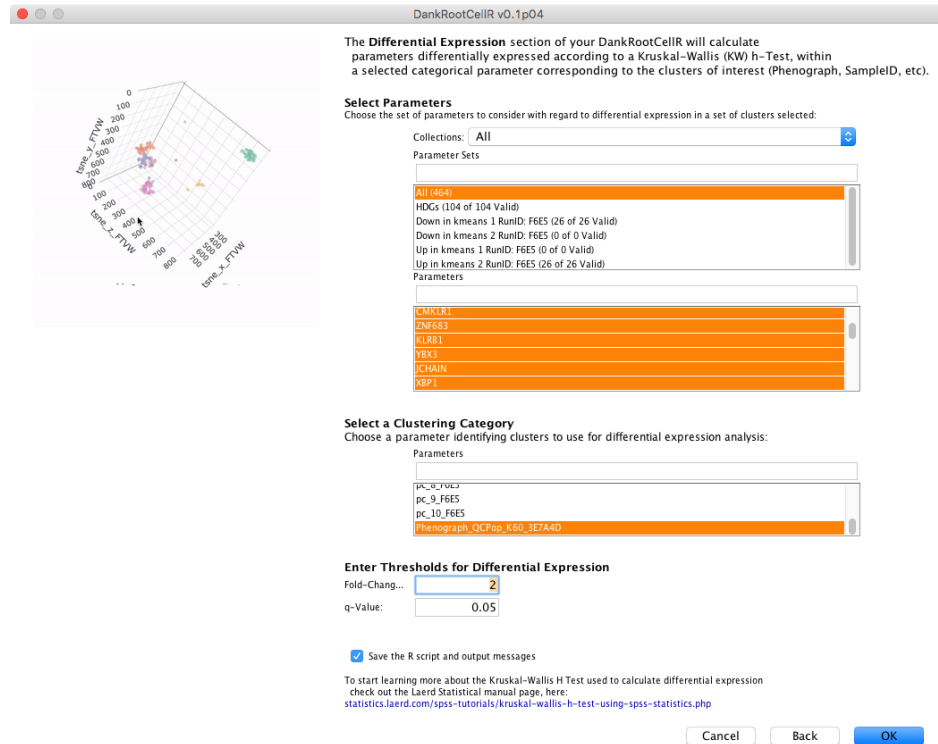
Note: If you want to avoid clustering and create monochrome 3D plots directly from the pipeline’s dimensionality reduction, set the Clustering Method to “None”.



- 3D plots** The 3D plots option is a great way to visualize your previously calculated, dimensionally reduced, plots in 3D. You will need to select the parameters to view for the X, Y, and Z axes. Optionally, you can also select categorical parameters to use for coloring the plot or to use as cell annotation during 3D mouse over. Ideal optional parameters for coloring or cell annotations would be SampleID or clustering parameters.



- **Differential Expression** The Differential Expression analysis workflow is another great feature that will allow you to calculate the differential gene expression of parameters with the click of a button. Before starting this analysis, you will need to run a unsupervised clustering with one of the options such as Phenograph or Xshift. The clustering category will need to be selected at the bottom of the dialog window in order to identify the clusters for differential expression analysis.



Known Issues

1. Choosing continuously expressed parameters in place of categorical parameters will cause issues with coloring, and values reported on mouse over. **Workaround:** Try to only include categorical parameters (or those with very small ranges) as categorical parameter choices.
2. Parameter naming from FlowJo will include only the \$P#N parameter naming keyword.
3. Not all clustering option combinations in the iCellR Pipeline workflow are expected to produce results, or quality clustering options. If only one or fewer clusters are generated in the pipeline, an interactive monochromatic 3D plot will be generated instead of the colorful version.
4. Clustering parameters chosen which don't represent continuous integer values will likely result in a cluster number not correlating to the values reported in FlowJo (e.g. FlowSOM).
5. Differential expression analysis can only be performed with the help of a clustering parameter, preventing its use for populations gated manually in SeqGeq. **Workaround:** create a concatenated population from populations of interest, thus creating a "SampleID" parameter which can be used for cluster identification in differential expression analysis.
6. The original iCellR package includes more functions than just DimRedux, DEG, and Interactive 3D plotting. We do hope to include some of the other functions from iCellR in future updates to the iCellR platform, stay tuned.

7. Researchers should not select the same categorical parameters as those selected for 3D plotting, this will cause the calculation to fail.

Leave us your feedback

Please write to flowjo@bd.com with any questions or concerns.

References

1. <https://github.com/rezakj/iCellR>