

How to Phenograph

Introduction

PhenoGraph is a clustering algorithm that robustly partitions high-parameter single-cell data into phenotypically distinct subpopulations. First, it constructs a nearest-neighbor graph to capture the phenotypic relatedness of high-dimensional data points and then it applies the Louvain graph partition algorithm to dissect the nearest-neighbor graph into phenotypically coherent subpopulations.

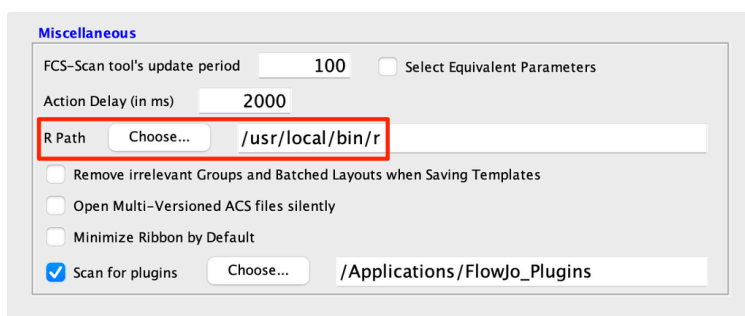
The PhenoGraph algorithm has been implemented as a plugin compatible with both FlowJo and SeqGeq analysis programs. As a result, this plugin will produce distinct subpopulations based on a derived “phenograph cluster number parameter”.

Please review FlowJo documentation for installing plugins: [Installing Plugins - FlowJo Documentation](#)

In order to install and build the required package for Phenograph you will need to download and install RTools which you can get as a free download: [RTools: Toolchains for building R and R packages from source on Windows](#) If you have a Mac you will need to install XQuartz from here: [XQuartz](#)

Download and Installation

1. Place the plugin .jar file in your Plugins folder, and direct FlowJo or SeqGeq 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. A common R path for Windows is “C:\Program Files\R\R-4.4.1\bin\x64” (for R version 4.4.1) and for Mac is “/usr/local/bin/r”.



3. Restart the (FlowJo or SeqGeq) application to pick up the new plugin.

Note The Phenograph plugin will now try to self-install when you run the plugin for the first time. If this does not work, try following step 4 below to install the dependencies.

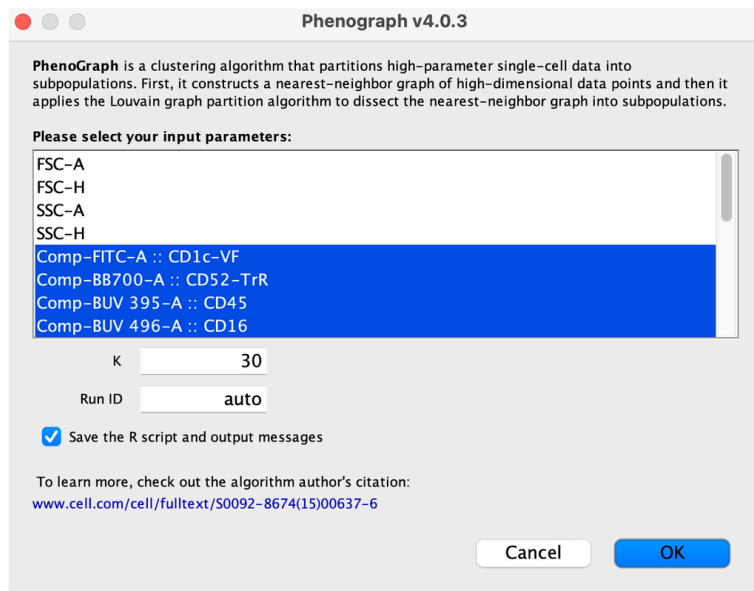
4. The R packages can be installed by typing (or copy/paste) the following in your R console:

```
1 install.packages(c('dplyr', 'viridisLite', 'magrittr', 'colourvalues', 'pheatmap', 'ggplot2', 'Rcpp', 'RANN',  
  'igraph', 'R6'))
```

Usage

To run the PhenoGraph plugin on a population (or sample),

1. Select the population of interest within the workspace
2. Go to the Workspace tab and select the PhenoGraph option from within the Plugins dropdown there. **Note** that the plugin will be unavailable (greyed out) if no population is selected.
3. Select the parameters and settings that you would like to use to run the PhenoGraph algorithm within the resulting plugin dialog:



- **Parameter Selector:** Which input parameters, such as FCS channels in FlowJo, principal components or genes or transcripts in SeqGeq, you want to run the algorithm on.
 - **K:** The number of nearest neighbours to be used for the nearest-neighbor graph to capture the phenotypic relatedness of high-dimensional data points. According to the original paper, PhenoGraph isn't typically very sensitive to this parameter as long as the values are reasonable. The authors have shown good results for K between 15 and 60 when clustering healthy immune cells, see figure 2B in Levine et al., 2015.
 - **Run ID:** An identifier used to name the created cluster id parameter. The "auto" value will create the name automatically based on the provided settings.
4. Once the plugin has finished calculating, it will create a new parameter capturing the PhenoGraph cluster ID along with gates, dissecting the input population into distinct subpopulations as clustered by PhenoGraph. In addition, PhenoGraph will export a file in its output folder with the resulting modularity value. Modularity is a value between -1 and 1 that is used as a score for the quality of the graph partitioning. Formal definition of modularity is in Newman and Girvan, 2004.

Leave us your feedback

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

References

1. Levine et al. Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis. Cell 162, 184–197, July 2, 2015, Elsevier Inc. [Data-Driven Phenotypic Dissection of AML Reveals Progenitor-like Cells that Correlate with Prognosis](#)
2. DiGiuseppe et al. PhenoGraph and viSNE facilitate the identification of abnormal T-cell populations in routine clinical flow cytometric data. Cytometry B Clin Cytom. 2017 Sep 2. doi: 10.1002/cyto.b.21588.
3. Newman and Girvan. Finding and evaluating community structure in networks. Physical Review E 69(2 Pt 2):026113 March 2004, DOI: 10.1103/PhysRevE.69.026113. [Finding and evaluating community structure in networks](#)
4. [GitHub - JinmiaoChenLab/Rphenograph: Rphenograph: R implementation of the PhenoGraph algorithm](#)