Human Peripheral Blood Phenotyping Panel Kit
Cytobank Guide to SPADE Analysis v 1.0

Cytobank guide for the identification of cell types from the Human Peripheral Blood Phenotyping Panel Kit. By the end of this guide, you will have learned the general principles for how to run and annotate a SPADE tree. In this example, you will do this for the 17-marker Human Peripheral Blood Phenotyping Panel Kit supplied by Fluidigm.
First Steps

Log into fluidigm.cytobank.org. You can register either using your OpenID account (Google, Yahoo) or password.

(1) Click to open the Fluidigm Cytobank Human PBMC Analysis Guide experiment, located in the Fluidigm Kits project. In the actions box,

(a) click on "Clone FCS files" and then

(b) click "OK" when asked if you desire to make your own copy of the example data set.

The page will verify that you have created a new experiment. There should be two fcs files. Click on "View Experiment Summary" as indicated by the orange arrow at the top left of the screen under “Actions.”

The Experiment Summary Page contains all of the information about the original experiment, including the Experiment Name, sharing information, and Illustrations that can be shared with other Cytobank users.

(2) Click on "Edit Experiment Details" under Actions at the top left. The following page will appear. You will be the default Primary Researcher and PI/Manager. You can assign these roles to other people here as well.

(3) Change the Experiment name by removing (clone) from the end and add “SPADE Tutorial - (your name)” at the beginning.

(4) Click the “Update Experiment” button to save these changes. You will be brought back to the Experiment Page.
Here are the Illustrations. Each person who has access to an experiment has their own Working Illustration. You can also save snapshots of your current working illustration. In order to run SPADE, we want to do a simple cleanup gate on our data to remove debris.

(5) Click on “Setup” under “Channels.” Recategorize the file starting with “Bead_cells_found” to “Other Controls.” Click “Return to Illustration.”

(6) Click on “Gate” under populations to proceed.

Here is the gating page. Change the default parameters on the plot to DNA (y) vs Cell Length (x) (also sometimes referred to as Event Length). Then draw a Singlet gate on the graph as shown in the example.

(7) Click on one of the gate buttons at the top of the window (i.e. polygon) and draw a singlet gate on DNA1 (Iridium-191) vs Cell Length on the active population. Name your gate and click “OK.”

(8) You can click “Check Gate” at the bottom right to see what percent you’ve gated on across all the files in this experiment.

(9) Once you are satisfied with your gates, hit “Apply and Return.” This will return you back to your Working Illustration. The example on Fluidigm Cytobank contains other gates that are available to you in the original gating strategy available online.
Running SPADE

At the top left of the screen, click “Back to Experiment”. Under “Actions,” select “Create SPADE Analysis.” A popup will appear. Name your spade run “Fluidigm Cytobank HuPBMC Guide.”

Here you have only a few things that you will have to fill out before starting your run. These include the following:

- Indicating the ideal number of nodes to cluster.
- What percent of the data would you like to preserve during downsampling (default is 10%)
- For which population do you want to run SPADE?
- What channels do you want to use to cluster your samples?
- (optional) Are there a set of baseline files that you want to use for fold change calculations?

(1) To simplify the SPADE tree, change the Target Number of Nodes for clustering to 100. You can also try the default (200).

(2) To simplify the number of cells being sampled, change the Absolute Number of cells to be 6000. You can also keep the default percent.

(3) Choose Singlets as the gate to be used for the SPADE Analysis by clicking on “Choose” in the Populations pane, then checking “Singlets”, and then clicking “Done”.

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(4) In Clustering Channels, click “Choose.”

(5) Select the following 15 Channels: CD19, CD4, CD8a, CD20, CD16, CD66, CD123, CD45, CD11c, CD14, CD27, CD45RA, CD3, CD38 and HLADR. You can do this quickly by the following steps by searching both CD and then HLADR in the dropdown menu. Click “All,” then click “Done.”

(6) You will see a green box below that says “When you are done setting up your SPADE parameters, click here to run the analysis.” After checking all of your Setup parameters, click the link to start your spade run.

A progress bar will start. This experiment usually takes approximately 10 minutes to run. Once it is complete, you will receive an email that it has finished.
Once your SPADE run has completed, you will be presented with a tree that is unannotated. It will look different from the one displayed.

You can select multiple nodes by either clicking and dragging your mouse to highlight nodes, or holding down shift and selecting individual nodes.
Annotation Step 1: Bubbling General Cell Types

Each node represents a cluster of events (e.g. cells). A larger node contains more events than a smaller node, and the color of the node represents a specific parameter, which allows for the distinguishing of positive and negative cells during the bubbling process. Bubbling is the process of manually identifying phenotypically similar nodes. Here is one example of a SPADE tree—your topology and bubbling may vary from this strategy.

(1) The first step is to bubble larger populations and those which do not require many markers. We give examples which are CD3+, CD20+, CD11c and CD16. These are meant as a guide—you may find it easier to select other channels for incremental bubbling.

(a) Below is an example 2D plot when the CD20+ cells are highlighted. Selecting nodes on the tree highlights the corresponding cells in the 2D plot. Multiple 2D plots can be popped out to help with bubbling by clicking “Pop-Out New Plot”.

(b)
Annotation Step 2: Bubbling Intermediate Cell Types

(2) The second step is to bubble slightly smaller populations. The examples included here are colored by the channels CD123 and CD14.
Annotation Step 3: More Intermediate Bubbling

(3) Here is more intermediate bubbling with the markers used to achieve the populations. Step 4 contains recognizable names for many of these populations, some of which were subsequently merged.

Example markers displayed include CD4 and CD8.
Annotation Step 4: A Fully Annotated Tree

(3) Your tree may have a different topology than the example, as well as a slightly different bubbling strategy. You can also increase the number of nodes for a SPADE tree to keep the topology more consistent, but the resulting nodes will have fewer cells.
Quick SPADE references:

<table>
<thead>
<tr>
<th>Action</th>
<th>Shortcut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change Sample/File Name</td>
<td>select left/right arrow keys</td>
</tr>
<tr>
<td>Change Parameter</td>
<td>select up/down arrow keys</td>
</tr>
<tr>
<td>Select multiple nodes</td>
<td>hold down shift, then click to drag the mouse and high-light/select groups of nodes. Then, use shift + click to select any remaining single nodes</td>
</tr>
<tr>
<td>Add/remove a bubble</td>
<td>click “Add Bubble” button</td>
</tr>
<tr>
<td>Select a bubble</td>
<td>click inside a bubble but not on a node</td>
</tr>
<tr>
<td>Move nodes/bubble</td>
<td>click on a node inside the bubble and drag your mouse</td>
</tr>
<tr>
<td>Rotate a bubble</td>
<td>select a bubble, then press ‘Z’. Drag the mouse to rotate. press ‘Z’ to stop rotation mode</td>
</tr>
<tr>
<td>Add a node to a bubble</td>
<td>click and drag the node into the bubble</td>
</tr>
<tr>
<td>Remove a node from a bubble</td>
<td>hold down shift, click the node and drag it outside of the bubble</td>
</tr>
</tbody>
</table>