SPADE Step-By-Step



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SPADE developer provides great instructions including screenshots

http://pengqiu.gatech.edu/software/SPADE/



Import pre-gated fcs files (live, singlet, gated on population to cluster) into SPADE

Use your fcs files or choose the "Exported FCS files" below. Files are located in the same PittBox as this ppt.



Import gated populations into SPADE

1. Click "Browse" and navigate to folder that contains gated fcs files

2. Select the folder and click "Close"

SPADE

SPADE			View_Edit_file_	_annotations	
Select working directory	The s	second	column is editable. Modify this column to give short anno	otation (name) for each file	
/Osers/iisaborgriesi/Desktop/cyt3-mac/z_collabs/winitey/off1316/exports_remain			filename	file short annot	
Browse		1	export_PsO_01041801_CD3_dump.fcs	export_PsO_0104	
		2	export_normal_lib1mg_CD3_dump.fcs	export_normal_lib1	
View FCS file list and edit file annotations: View File List					
Setup SPADE algorithm parameters					
View / update algorthm parameters					
Run SPADE analysis					
Compute local densities for each file					
One click for all Pool selected files					
Clustering+MST+layout+upsample					
				2.	Close
Visualize results					
View resulting SPADE tree					

Setup SPADE parameters



3. Click "View/update algorithm parameters"

Setup SPADE parameters

View_Edit_S	PADE_parameters
Markers used to build SPADE tree	Files used to build SPADE tree
Overlapping markers that are not used	Files NOT used to build SPADE tree
Compensation option	Max allowable cells in pooled downsampled data: 50000
Ignore compensation	Outlier and Target Densities (OD and TD)
Apply compensation matrix in FCS header	Outlier de 1 th percentile of local densities (LDs) of all cells
No Transformation	Target density: percentile of LDs 3
Arcsinh with cofactor 150	• fixed number of cells remain 24000
Arcsinh with above cofactor, followed by 0-mean-1-var	- Clustering parameter
Local density calculation parameters	Algorithm: • K-means
Neighborhood size = mediam min dist * 5	Agglomerative
Local density approximation factor: 1.5	Number of desired clusters: 35

4. Select markers for building SPADE tree (move left >> right)

5. Select files to use for building SPADE tree (ditto)

Setup SPADE parameters

Markers used to build SPADE tree		Files used to build SPADE tree	
Overlapping markers that are not used	Overlapping markers used for SPADE tree TCR alpha beta	Files NOT used to build SPADE tree	Files used to build SPADE tree
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Compensation option		Max allowable cells in pooled d	ownsampled data: 50000
Apply compensation matrix in	FCS header	Outlier de 1 dens	th percentile of local ities (LDs) of all cells
No Transformation Arcsinh with cofactor	150	Target density: percenti fixed nu	le of LDs 3 mber of cells remain 24000
-Local density calculation parameters - Neighborhood size = mediam	min dist * 5	Clustering parameter Algorithm: • K-means Agglomerative	9.

- 6. Select "Ignore compensation" since we are using compensated data from FlowJo
- 7. Arcsinh transform, cofactor 150
- 8. Assign target density such that a fixed number of cells survive the downsampling process
- 9. Set desired number of clusters

Run SPADE analysis

Users/lisaborghesi/Deskt	op/cyt3-mac/z_Collabs/Whi	tley/011318/exports_re
		Browse
View FCS file list an	d edit file annotations:	View File List
tup SPADE algorithm pa	rameters	
Vie	w / update algorthm param	eters
IN SPADE analysis	10.	
	Compute local densi	ties for each file
One click for all	Pool select	ed files
	Clustering+MST+la	yout+upsample

10. Today we'll sequentially walk through each step in the algorithm.

- a) Compute local densities for each file
- → Fast. Feedback is "100% Done"
- b) Pool selected files
- → Takes a bit longer. Feedback is "Done!"
- c) Clustering
- → Fast. Feedback is "Done"

Run SPADE analysis



11. View resulting SPADE tree

Color tree by marker

Show node/cluster index Edit SPADE tree layout Selected nodes: Scale span of selected nodes: Acta select nodes: Change node size: Change node size: SPADE tree annotation Auto Suggest Annotation Auto Suggest Annotation View Annotations Remove Auto Suggest Annotation No show Show all Show selected Show selected Show selected Show selected Show selected Show selected Ref files for ratio CD4 CD4 CD5 Ref files for ratio CD4 CD5 Ref files for ratio CD4 CD5 Ref files for ratio CD4 CD5 Ref files for ratio CD4 CD5 Ref files for ratio CD4 CD5 CD6 Ref files for ratio CD4 CD6 CD6 Ref files for ratio CD4 CD5 CD6 Ref files for ratio CD4 CD6 Ref files for ratio CD4 CD6 CD6 Ref files for ratio CD4 CD6 Ref files for ratio CD4 CD5 CD6 Ref files for ratio CD4 CD4 CD6 Ref files for ratio CD4 CD5 CD6 Ref files for ratio CD4 CD6 Ref files for ratio CD4 CD4 CD6 Ref files for ratio CD4 CD4 CD4 CD6 Ref files for ratio CD4 CD4 CD4 CD4 CD4 CD4 CD4 CD4	show tree in new wind	dow	Arch Lav	vout	Spring	RadioExpar
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 \rightarrow Circle size proportional to pop frequency



- Check the overlay box
- Select marker (e.g., TCRab)
- Select file (e.g., pooled)
- Select "expr" = median fluor intensity of cells in the cluster

Compare samples



Or compare samples:

<u>Expr</u> = median fluor intensity of cells in that cluster

<u>Ratio</u> = the difference b/w two samples

<u>Cell freq</u> = freq. of cells in cluster

Compare samples

Select a marker:	Select a file	F	Ref files for ratio
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esults to PS, GML, TXT,	-CS formats		
Tree colored by	y markers	Tree	colored by CellFreq
Tree to GM	L & TXT	Node/Anno	ot expr & CellFreq to TXT

Export SPADE trees or files

Annotate SPADE tree (unbiased)

Show node/cluster index				
Selected nodes:				
			ad	ld to annotation
Scale span of selec	ted nodes:	[F]		
Rotate select nodes:	- EFE -			
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- 1. Select "Auto Suggestion Annotation"
- 2. In popup window, accept/reject proffered partition



Annotations (unbiased)



Changes in population frequency PsO_Stelara relative to Normal



References

Reviews

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Algorithms

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