# Start interactive job

bsub -W 8:00 -n 12 -M 128000 -Is bash

module load anaconda3

source /usr/local/anaconda3-2020/etc/profile.d/conda.sh

conda activate /data/salomonis2/LabFiles/Kyle/Env/pyInfinityFlow\_pypi

# “pip show pyInfinityFlow” to check version

# get backbone annotation from any file

pyInfinityFlow-list\_channels --fcs\_file "export\_Grimes\_human\_marrow-Infinity\_B\_12\_6\_22-Plate 1-Group\_001-A1 CD2 PE\_Unmixed\_live.fcs" --add\_user\_defined\_names True

#copy output to excel and split columns based on “:”

Delete the FSA and SCC, and PE

A screenshot of a computer

Description automatically generated

Save as “backbone\_anno.csv”

#list all files in the folder

ls -1> list.txt

#copy the file names and split columns to list antigen(Name), “FJComp-PE-A” as (Channel), delete (isoform)

Table

Description automatically generated with low confidence

#save as “infinity\_marker\_anno.csv”

#if events in file is less than 10k, change “--n\_events\_combine 0”

#run pyinifinity using default settings

pyInfinityFlow --data\_dir /data/salomonis-archive/Infinity\_Grimes/01\_2023/live\_FCS/ \

--out\_dir /data/salomonis-archive/Infinity\_Grimes/01\_2023/live\_FCS/infinity\_output/ \

--backbone\_annotation /data/salomonis-archive/Infinity\_Grimes/01\_2023/live\_FCS/backbone\_anno.csv\

--infinity\_marker\_annotation /data/salomonis-archive/Infinity\_Grimes/01\_2023/live\_FCS/infinity\_marker\_anno.csv\

--use\_logicle\_scaling True \

--normalization\_method None \

--n\_events\_train 0 \

--n\_events\_validate 0 \

--ratio\_for\_validation 0.5 \

--separate\_backbone\_reference None \

--n\_events\_combine 0 \

--n\_final 0 \

--add\_umap True \

--find\_clusters True \

--find\_markers True \

--make\_feature\_plots True \

--use\_pca True \

--n\_pc 15 \

--n\_pc\_plot\_qc 50 \

--save\_h5ad True \

--save\_feather True \

--save\_file\_handler True \

--save\_regression\_models True \

--verbosity 3 \

--n\_cores 12