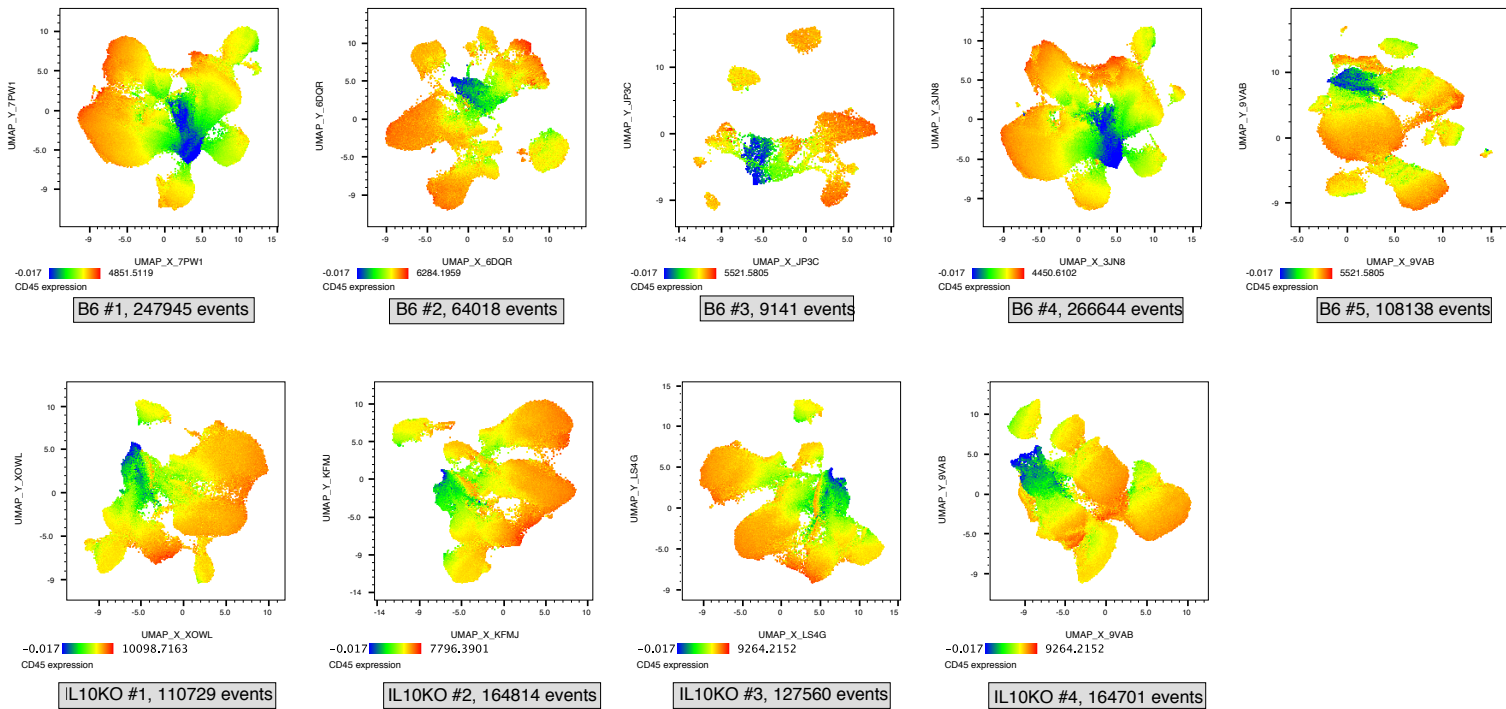


# Exploring the UMAP plugin in FlowJo

Abby Kimball, Clambey Lab 05/22/19

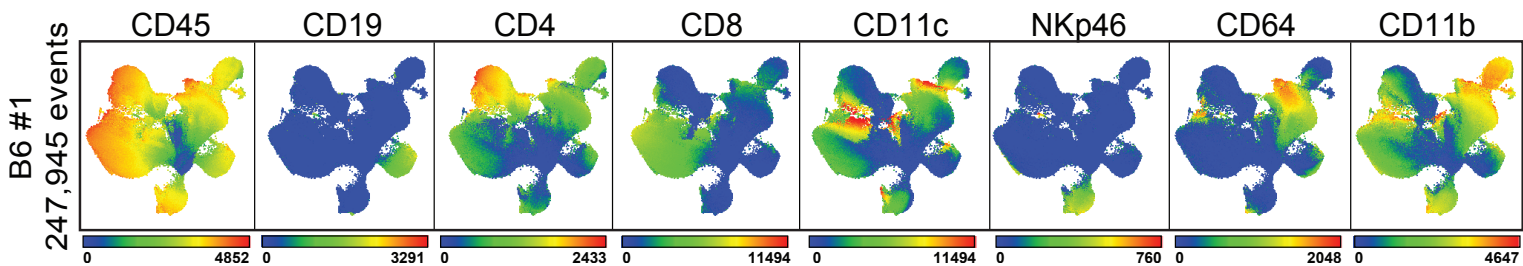
# All 9 UMAP plots

- Each file was analyze separately, all events were included, all clustered on relevant markers, and distance function “Euclidean”, nearest neighbors: 15, and minimal distance: 0.5.



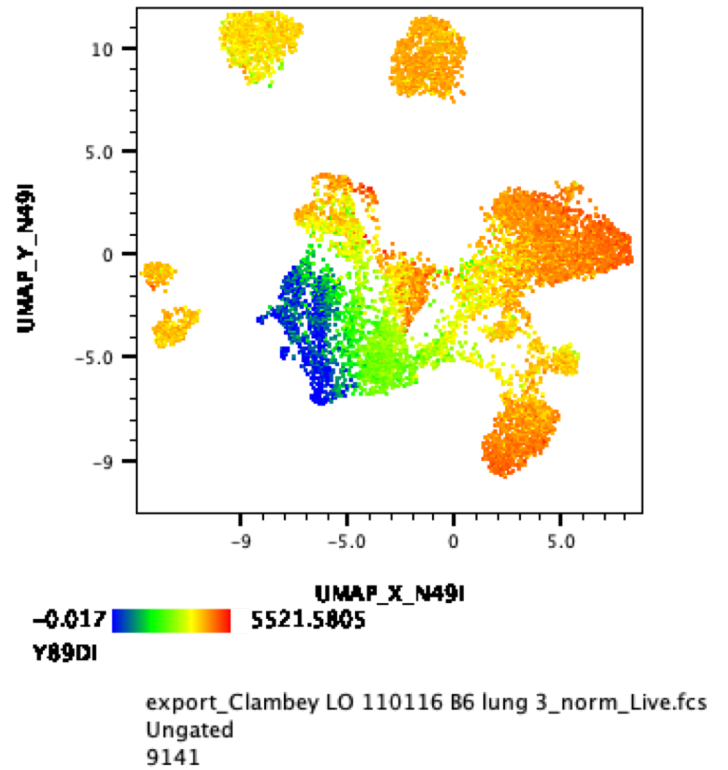
## B6 #1 colored by lineage markers

### UMAP in FlowJo\*

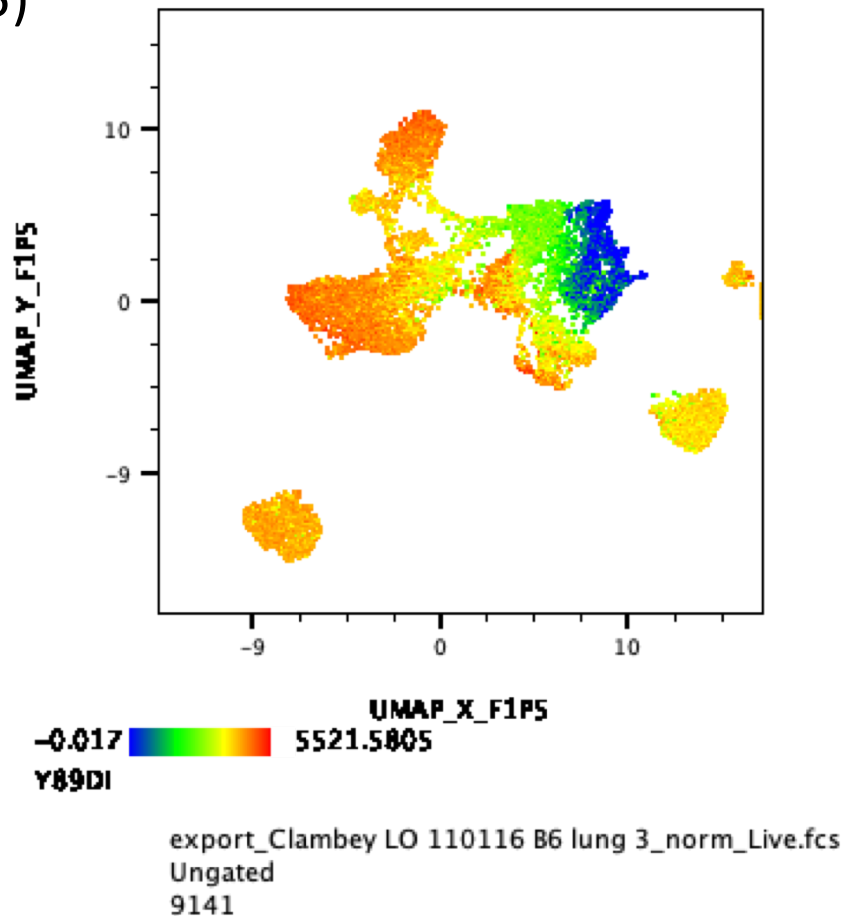


# Is UMAP reproducible?

- B6 #3 UMAP #1 (N49I)



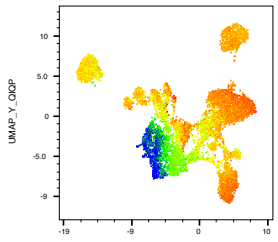
- B6 #3 UMAP #2 (F1P5)



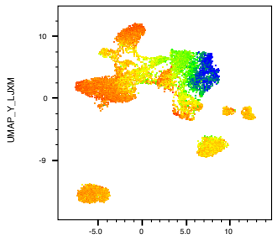
# B6 #3 UMAP iterations with all the possible distance functions

- Euclidean – QIQP
- Manhattan – LJXM
- Chebyshev – NG9Y
- Minkowski– XFVO
- Canberra – Q4FC **ERROR, FAILED**
- Braycurtis – 7H74 **ERROR, FAILED**
- Mahalanobis – BCGQ **ERROR, FAILED**
- Wminkowski – N6K4 **ERROR, FAILED**
- Seclidean – C9ER **ERROR, FAILED**
- Cosine – 8WI2 **ERROR, FAILED**
- Correlation - KHM1 **ERROR, FAILED**
- Haversine - N9Y1 **ERROR, FAILED**
- Hamming – V492
- Jaccard – APFR **ERROR, FAILED**
- Dice – 5U1M **ERROR, FAILED**
- Russelrao – 3JTI **ERROR, FAILED**
- Kulsinski – GRU0 **ERROR, FAILED**
- Rogerstanimoto – BOT8 **ERROR, FAILED**
- Sokalmichener – DV1E **ERROR, FAILED**
- Sokalsneath – 2ZK3
- Yule – JT53 **ERROR, FAILED**

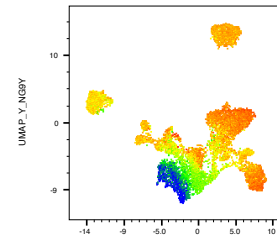
# B6 #3 UMAP iterations with all the possible distance functions



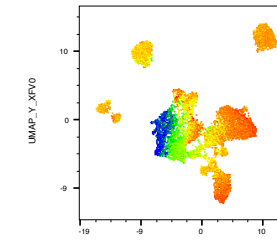
UMAP\_X\_QIQP  
5521.5805  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Euclidean  
9141



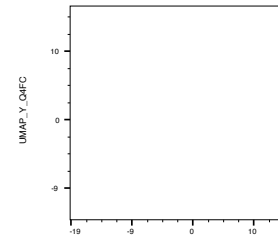
UMAP\_X\_LXXM  
5065.3081  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Manhattan  
9141



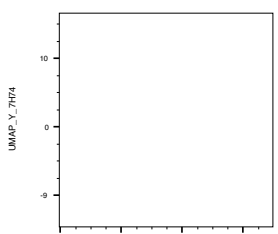
UMAP\_X\_NGBY  
5521.5805  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Chebyshev  
9141



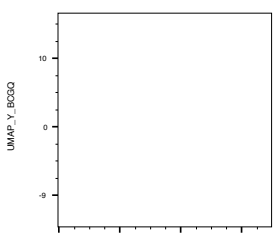
UMAP\_X\_XFV0  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Minkowski  
9141



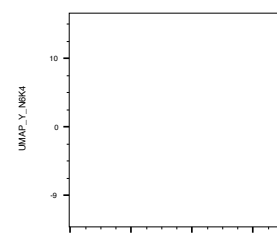
UMAP\_X\_Q4FC  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Canberra - FAILED  
9141



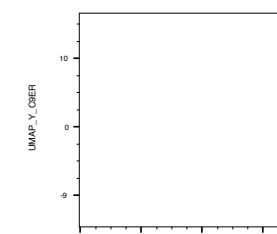
UMAP\_X\_7H74  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Braycuris - FAILED  
9141



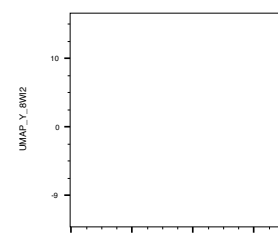
UMAP\_X\_BCGQ  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Mahalanobis - FAILED  
9141



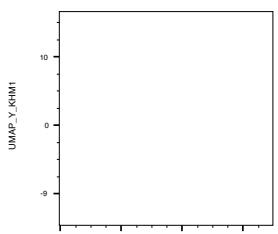
UMAP\_X\_N6K4  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Wminkowski - FAILED  
9141



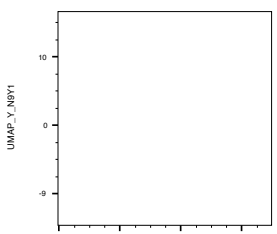
UMAP\_X\_C6ER  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Seuclidean - FAILED  
9141



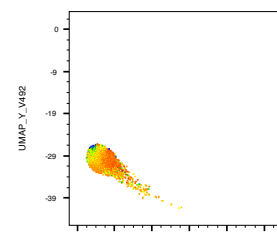
UMAP\_X\_8Wl2  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Cosine - FAILED  
9141



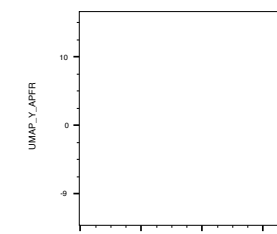
UMAP\_X\_KHM1  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Correlation - FAILED  
9141



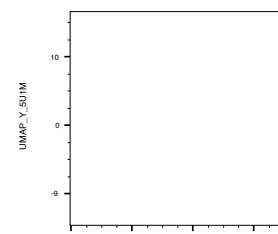
UMAP\_X\_N9Y1  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Haversine - FAILED  
9141



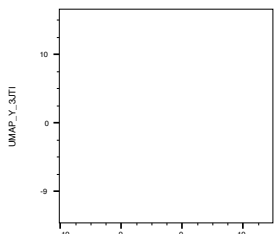
UMAP\_X\_V492  
4262.7591  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Hamming  
9141



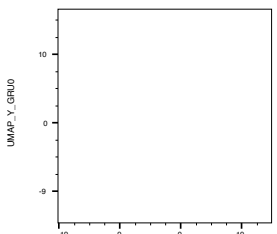
UMAP\_X\_AFPR  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Jaccard - FAILED  
9141



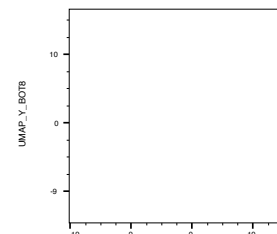
UMAP\_X\_5U1M  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Dice - FAILED  
9141



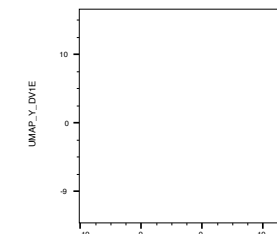
UMAP\_X\_3JTI  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Russelrao - FAILED  
9141



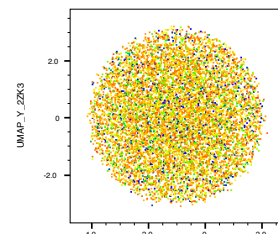
UMAP\_X\_GRU0  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Kulsinski - FAILED  
9141



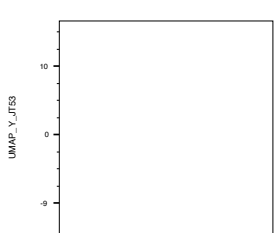
UMAP\_X\_BOT8  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Rogerstanimoto - FAILED  
9141



UMAP\_X\_DV1E  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Sokalmichener - FAILED  
9141



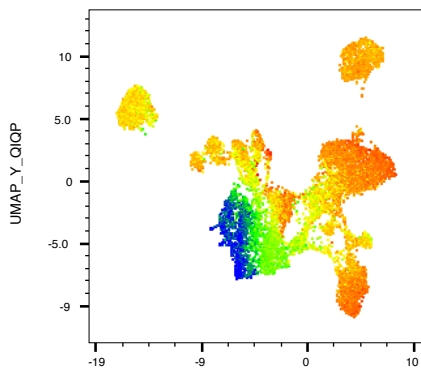
UMAP\_X\_2ZK3  
5521.5805  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Sokalneath  
9141



UMAP\_X\_JTS3  
4450.6102  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Yule - FAILED  
9141

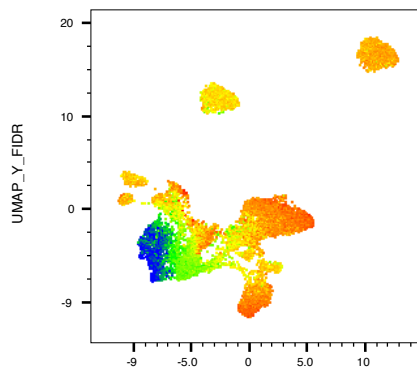
# B6 #1 UMAP iterations with various nearest neighbors #1-100

- Euclidean, neighbor #: 15 – QIQP
- Euclidean, neighbor #: 50 – FIDR
- Euclidean, neighbor #: 99 – AN6V **ERROR, FAILED**
- Euclidean, neighbor #: 0 – FUVQ **ERROR, FAILED**
- Euclidean, neighbor #: 5 – D4WN
- Euclidean, neighbor #: 1 – N3T1 **ERROR, FAILED**



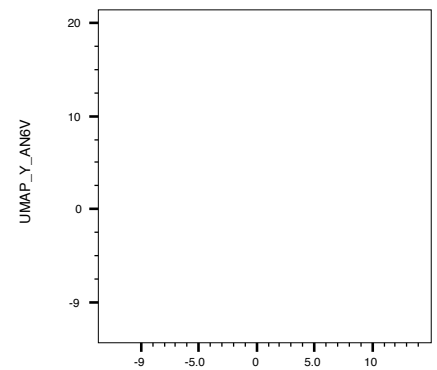
UMAP\_X\_QIQP  
-0.017 5521.5805  
Y89Di

export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Euclidean, neighbor #: 15  
9141



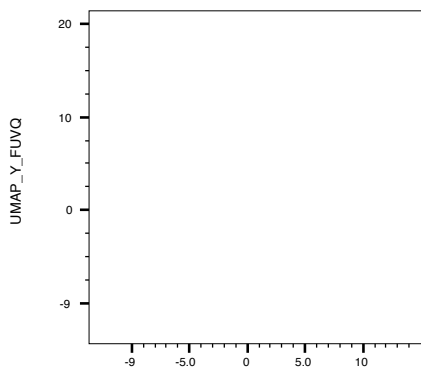
UMAP\_X\_FIDR  
-0.017 5521.5805  
Y89Di

export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Euclidean, neighbor #: 50  
9141



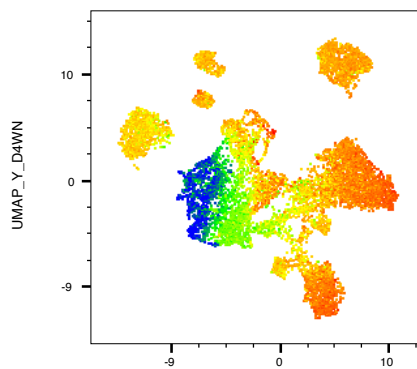
UMAP\_X\_AN6V  
-0.017 5521.5805  
Y89Di

export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Euclidean, neighbor #: 99 FAILED  
9141



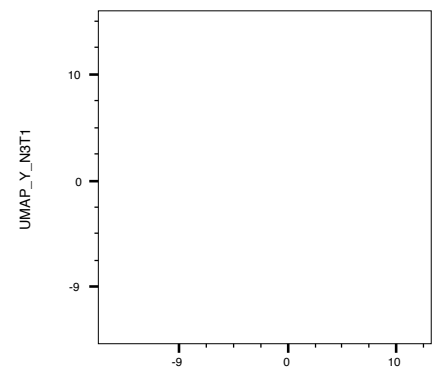
UMAP\_X\_FUVQ  
-0.017 5521.5805  
Y89Di

export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Euclidean, neighbor #: 0, FAILED  
9141



UMAP\_X\_D4WN  
-0.017 5065.3081  
Y89Di

export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Euclidean, neighbor #: 5  
9141

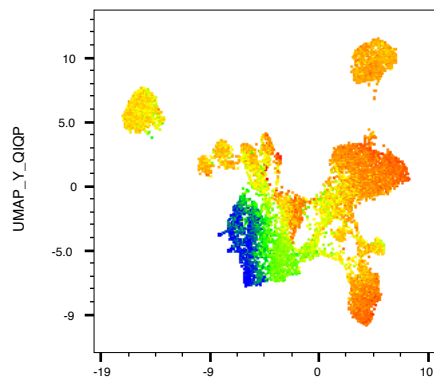


UMAP\_X\_N3T1  
-0.017 5065.3081  
Y89Di

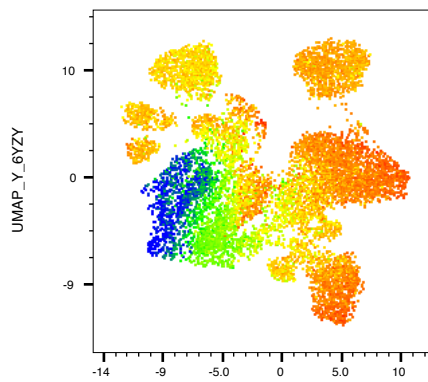
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Euclidean, neighbor #: 1 FAILED  
9141

# B6 #1 UMAP iterations with various min distance value = 0.1-0.99

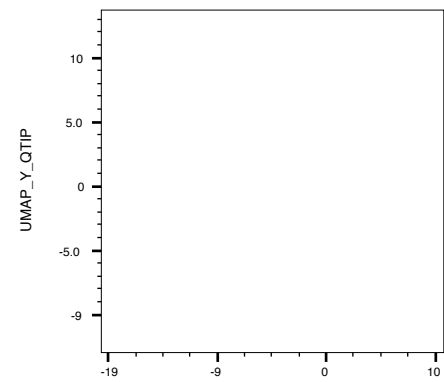
- Min distance = 0.5: – QIQP
- Min distance = 0.1: – 6YZY
- Min distance = 0.99: – QTIP **\*ERROR\***



UMAP\_X\_QIQP  
-0.017 5521.5805  
Y89Di  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Min distance 0.5  
9141



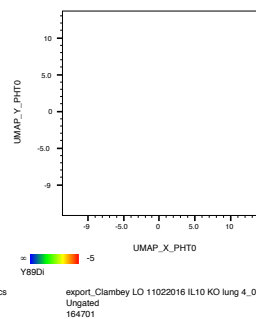
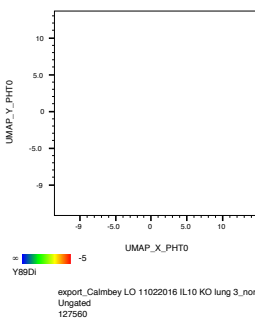
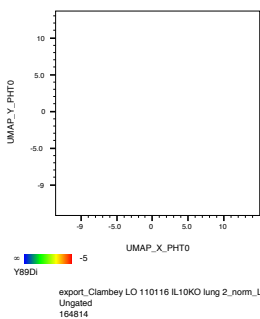
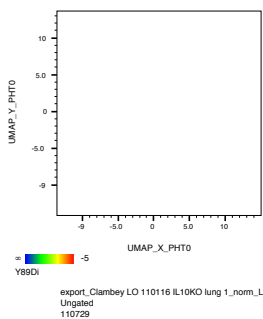
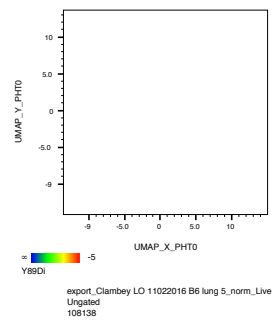
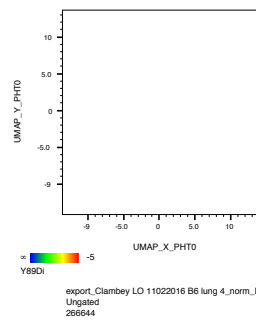
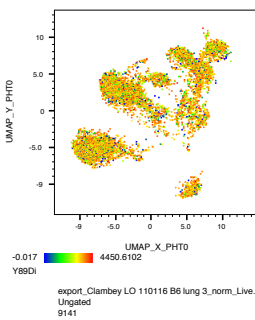
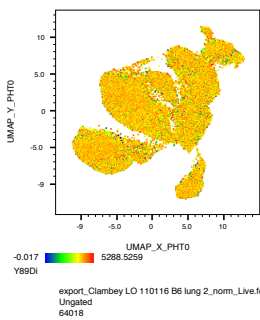
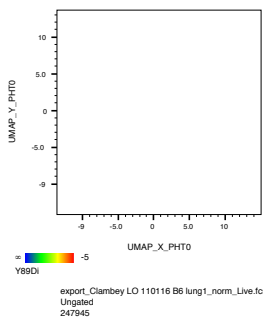
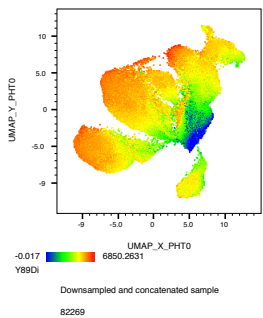
UMAP\_X\_6YZY  
-0.017 5521.5805  
Y89Di  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Min distance: 0.1  
9141



UMAP\_X\_QTIP  
-0.017 5521.5805  
Y89Di  
export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Min distance 0.99 FAILED  
9141

# How can you make a common UMAP plot between samples?

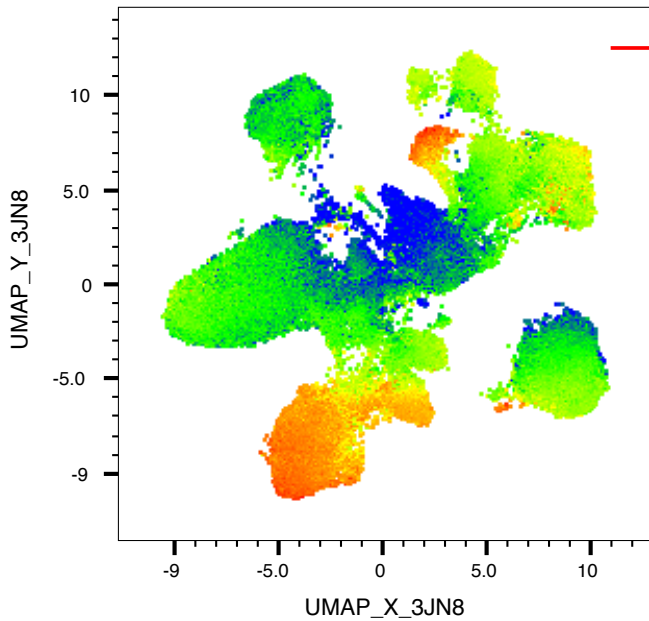
- I tried concatenating all of the available events (1,263,690 events total) and running a UMAP on it, but after 2 hours of crunching it froze and then crashed my computer.
- I downsampled each of the 9 files (9,141 events from each), concatenated, and then ran a UMAP analysis (82,269 events). I then applied this UMAP (PHT0) to all of the 9 samples. This only worked for samples with less than the concatenated # of events (i.e. B6 #2 & #3)






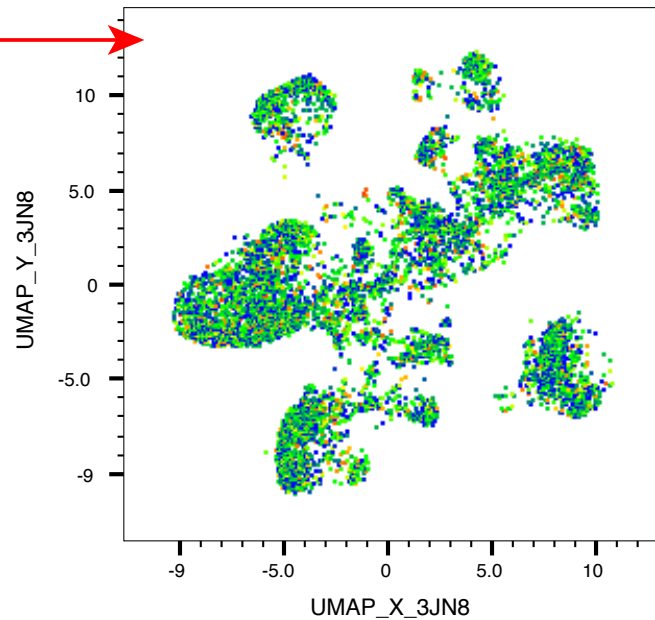
# Can you apply a UMAP generated by one sample to another sample?


UMAP Analysis 3JN8 was calculated using 64,018 events from B6 #2.  
The UMAP axes were then applied to B6 #3.



-0.017  2433.4182  
CD4 Expression

**B6 #2, 64018 events**

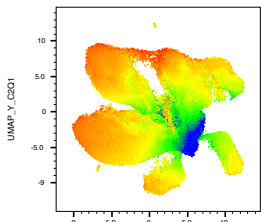


-0.017  2540.654  
CD4 Expression

**B6 #3, 9141 events**

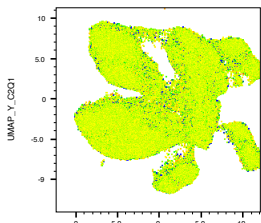
# How can you make a common UMAP plot between samples?

- I concatenated all of the available events (1,263,690 events total) and then downsampled to 266644 events (# of events that are the same as the highest sample) and then ran a UMAP and applied that UMAP to all of the files. It “worked”, but it doesn’t look right. Also note that I always get this warning, so I don’t think this is a viable option.



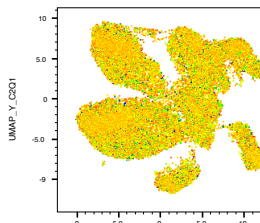
UMAP\_X\_C2Q1  
-0.017 6561.1276  
Y89Di

export\_concat\_1\_allEventsdown.Pop.fcs  
Ungated  
266644



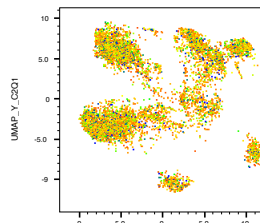
UMAP\_X\_C2Q1  
-0.017 11493.5047  
Y89Di

export\_Clambey LO 110116 B6 lung1\_norm\_Live.fcs  
Ungated  
247945



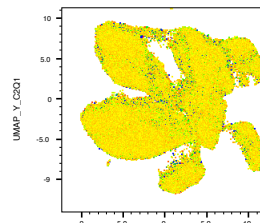
UMAP\_X\_C2Q1  
-0.017 6561.1276  
Y89Di

export\_Clambey LO 110116 B6 lung 2\_norm\_Live.fcs  
Ungated  
64018



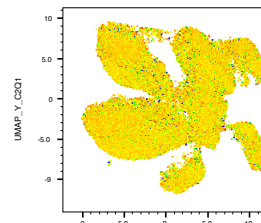
UMAP\_X\_C2Q1  
-0.017 5065.3081  
Y89Di

export\_Clambey LO 110116 B6 lung 3\_norm\_Live.fcs  
Ungated  
9141



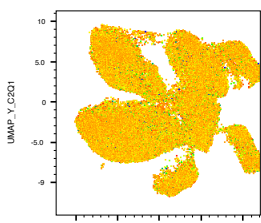
UMAP\_X\_C2Q1  
-0.017 4282.7591  
Y89Di

export\_Clambey LO 11022016 B6 lung 4\_norm\_Live.fcs  
Ungated  
266644



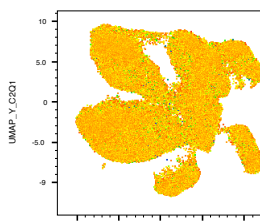
UMAP\_X\_C2Q1  
-0.017 5764.9053  
Y89Di

export\_Clambey LO 11022016 B6 lung 5\_norm\_Live.fcs  
Ungated  
106138



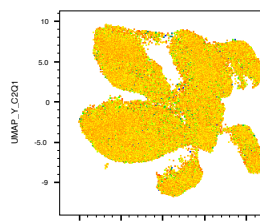
UMAP\_X\_C2Q1  
-0.017 6284.1959  
Y89Di

export\_Clambey LO 110116 IL10KO lung 1\_norm\_Live.fcs  
Ungated  
110729



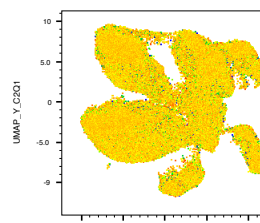
UMAP\_X\_C2Q1  
-0.017 5521.5805  
Y89Di

export\_Clambey LO 110116 IL10KO lung 2\_norm\_Live.fcs  
Ungated  
164814



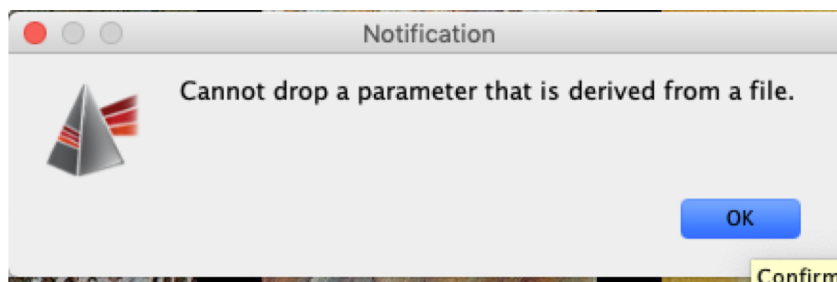
UMAP\_X\_C2Q1  
-0.017 7152.1403  
Y89Di

export\_Clambey LO 11022016 IL10 KO lung 3\_norm\_Live.fcs  
Ungated  
127560



UMAP\_X\_C2Q1  
-0.017 7467.3205  
Y89Di

export\_Clambey LO 11022016 IL10 KO lung 4\_01\_norm\_Live.fcs  
Ungated  
164701



# Analysis Time

- Doing over 1 million events took over 2 hours and froze and ultimately crashed my computer.
- 10k events took under a minute