Demo of EPP on OMIP-047 (HiD Phenotypic Characterization of B Cells) samples

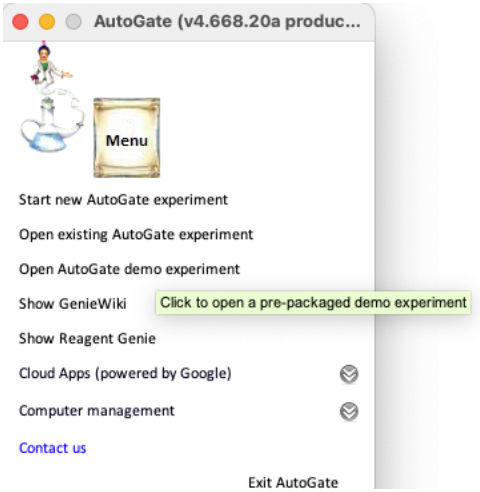
Version 1, November 2021

This demo teaches how to compare AutoGate's fully automatic gating to the cell types defined in OMIP's published gating strategy - http://cgworkspace.cytogenie.org/Tutorials/omipB.png
For reference, full publication is available here - https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.a.23488

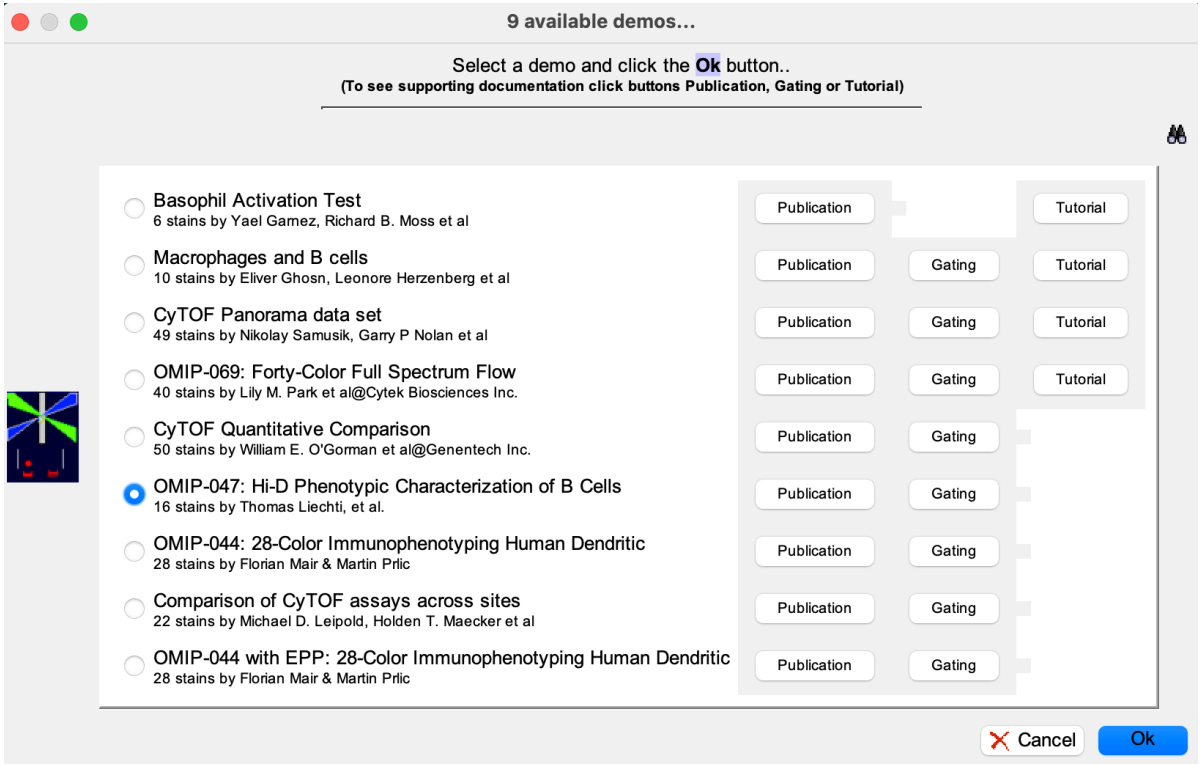
This document describes how to start in any position of a conventional gating hierarchy and run unsupervised automatic gating (EPP) and then compare the results between it and any other gating method AutoGate's HiD matching tools and highlighting tools.

1 Set up the experiment

1.1 Click "Open AutoGate demo experiment" on the main window



AutoGate responds by showing you the below list of demo experiments available as of November 2021



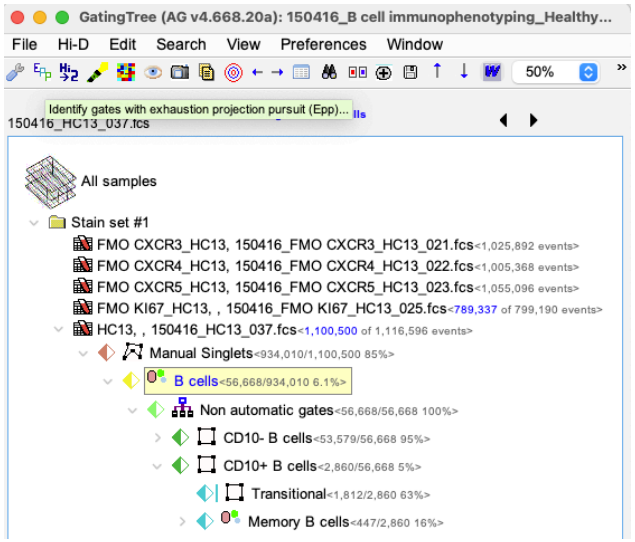
1.2 Pick OMIP-44: 28 Color Immunophenotyping Human Dendritic

AutoGate responds by opening up the GatingTree window on the publication's 4 full stained samples. The bundled demo has already completed the setup plus the replication of the published manual gating hierarchy for one sample. This starting point allows you to

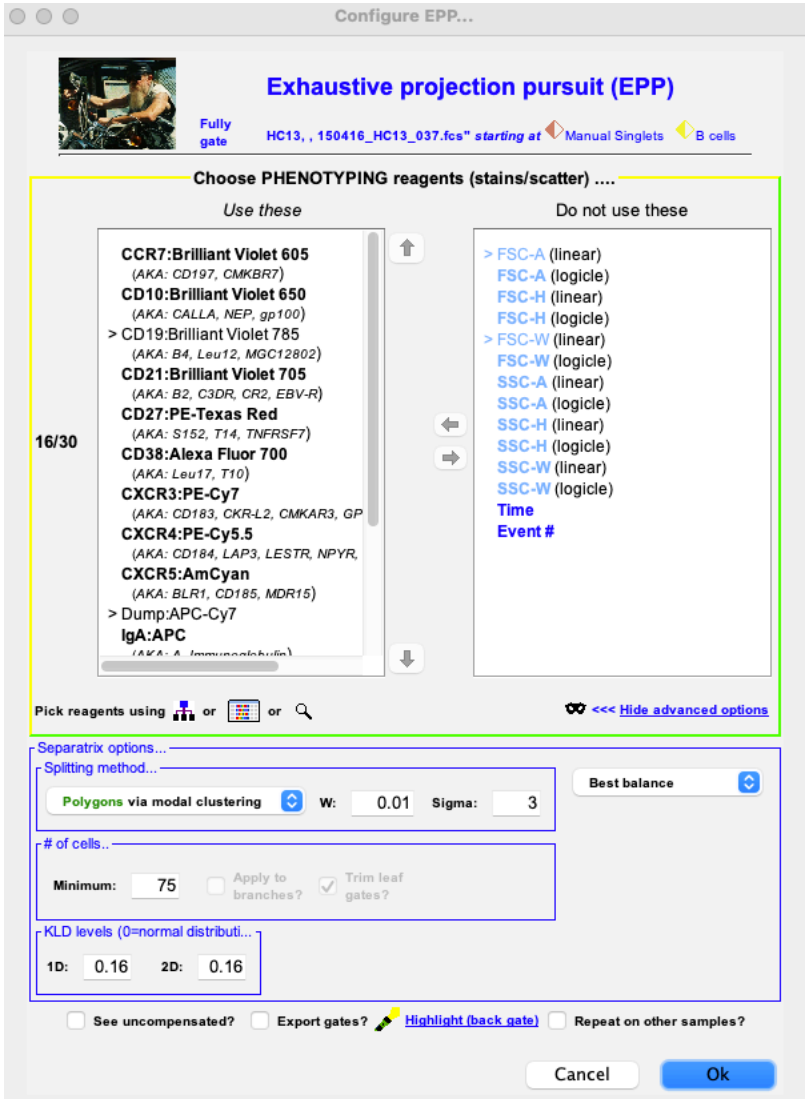
- Begin a fully automated analysis playing with AutoGate's fully automatic tools
  or
- Continue with a conventional analysis by dragging and dropping the provided gating **hierarchy** to other samples

2 Run a fully automated unsupervised analysis with exhaustive projection pursuit (EPP)

2.1 Select **B cells** in the gating tree and then click the toolbar's Epp button.



2.2 AutoGate responds by bringing up the following EPP configuration screen.


Configure EPP...
Exhaustive projection pursuit (EPP)
Fully gate
HC13, , 150416_HC13_037.fcs" starting at Manual Singlets B cells
Choose PHENOTYPING reagents (stains/scatter) ....
Use these
Do not use these
16/30
CCR7:Brilliant Violet 605
(AKA: CD197, CMKBR7)
CD10:Brilliant Violet 650
(AKA: CALLA, NEP, gp100)
> CD19:Brilliant Violet 785
(AKA: B4, Leu12, MGC12802)
CD21:Brilliant Violet 705
(AKA: B2, C3DR, CR2, EBV-R)
CD27:PE-Texas Red
(AKA: S152, T14, TNFRSF7)
CD38:Alexa Fluor 700
(AKA: Leu17, T10)
CXCR3:PE-Cy7
(AKA: CD183, CKR-L2, CMKAR3, GP
CXCR4:PE-Cy5.5
(AKA: CD184, LAP3, LESTR, NPYR,
CXCR5:AmCyan
(AKA: BLR1, CD185, MDR15)
> Dump:APC-Cy7
IgA:APC
> FSC-A (linear)
FSC-A (logicle)
FSC-H (linear)
FSC-H (logicle)
> FSC-W (linear)
FSC-W (logicle)
SSC-A (linear)
SSC-A (logicle)
SSC-H (linear)
SSC-H (logicle)
SSC-W (linear)
SSC-W (logicle)
Time
Event #
Pick reagents using or or
<<< Hide advanced options
Separatrix options...
Splitting method...
Polygons via modal clustering
W: 0.01
Sigma: 3
Best balance
# of cells..
Minimum: 75
Apply to branches?
Trim leaf gates?
KLD levels (0=normal distributi...
1D: 0.16
2D: 0.16
See uncompensated?
Export gates?
Highlight (back gate)
Repeat on other samples?
Cancel
Ok

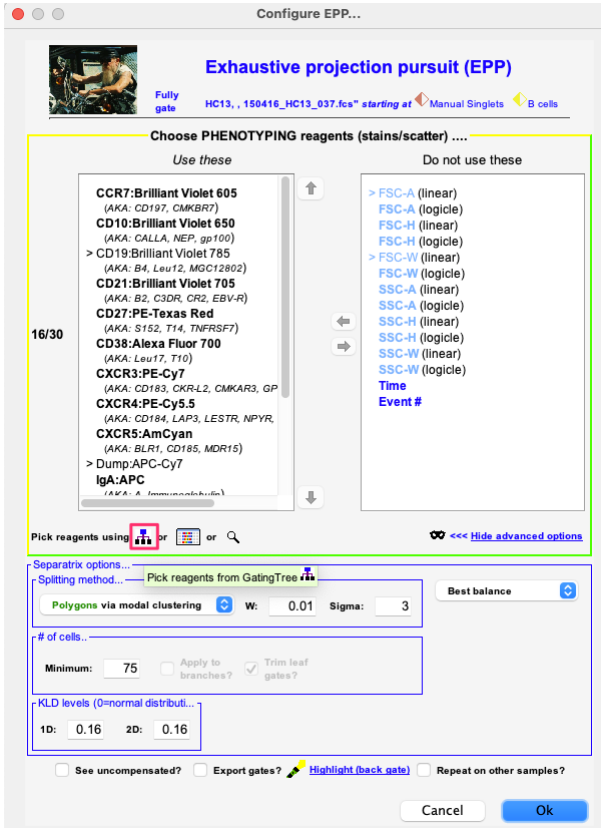
2.3 Specify the parameter for EPP to gate on

To effectively compare EPP results to the published manual results for a specific sub hierarchy we need to identify both the parameters that

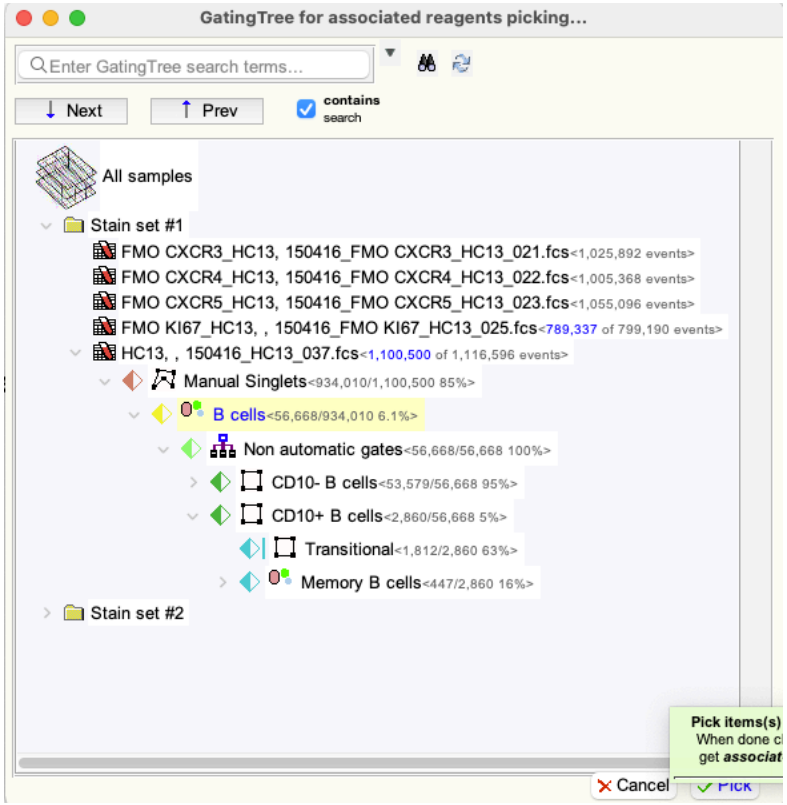
1. Were used for the manual analysis
2. Those that look informative for the same cell types

2.4 Point EPP at parameters used manually for **B cells**

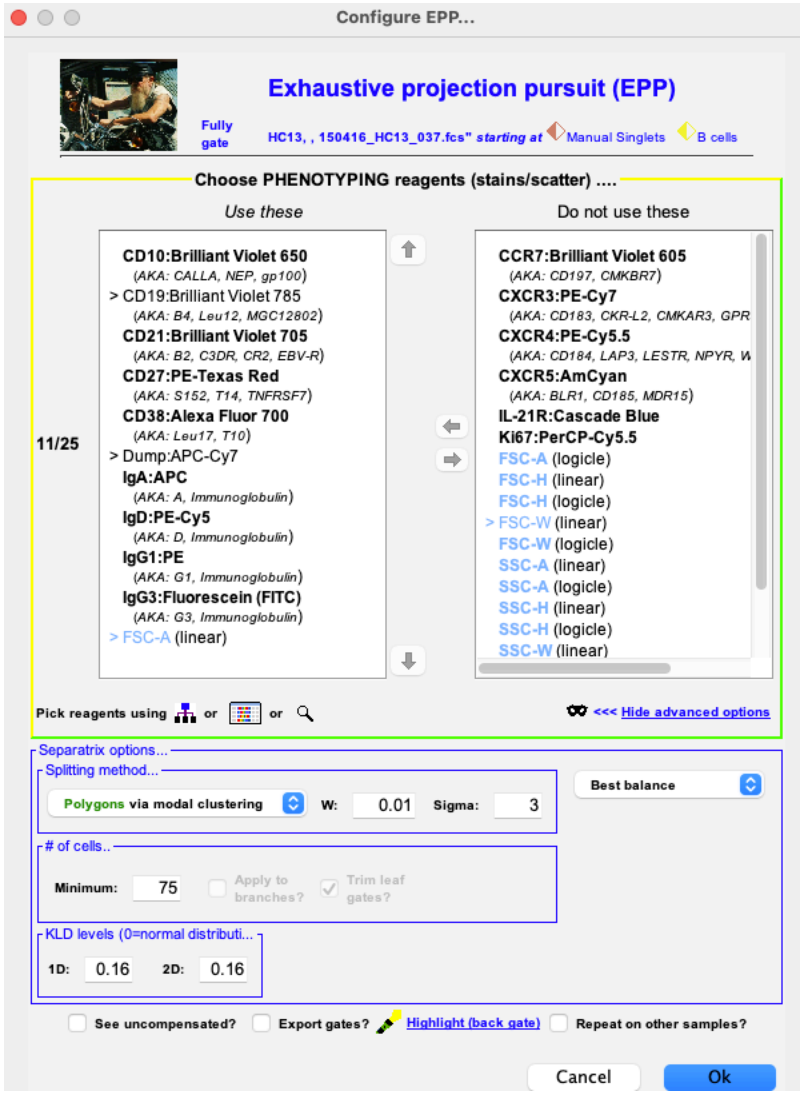
2.4.1 Click on the tree icon under the 2 parameter lists



2.4.2 Pick **B cells** in the gating tree

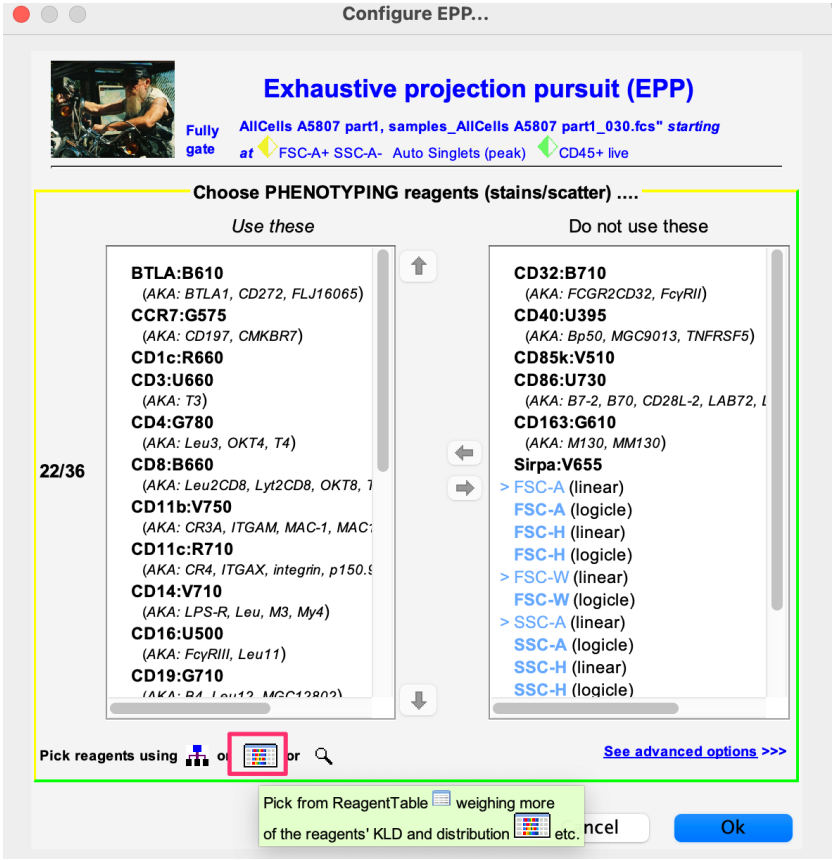


AutoGate populates the left list with the parameters that the OMIP investigators used in this sub hierarchy of their gating tree.



3 Point EPP at other parameters that appear informative for B cells

4 Click on the table icon under the 2 parameter lists



AutoGate shows a table of reagents with those currently used by EPP selected in a gold color.

5 Select parameters that have high KLD but are not already selected

Ensure that the reagents are sorted by highest to lowest KLD (Kullback Leibler divergence). KLD scores from 0 to 1 measure who closely a data distribution resembles the normal distribution. 0 is most resemblance and thus least interesting/informative structure.

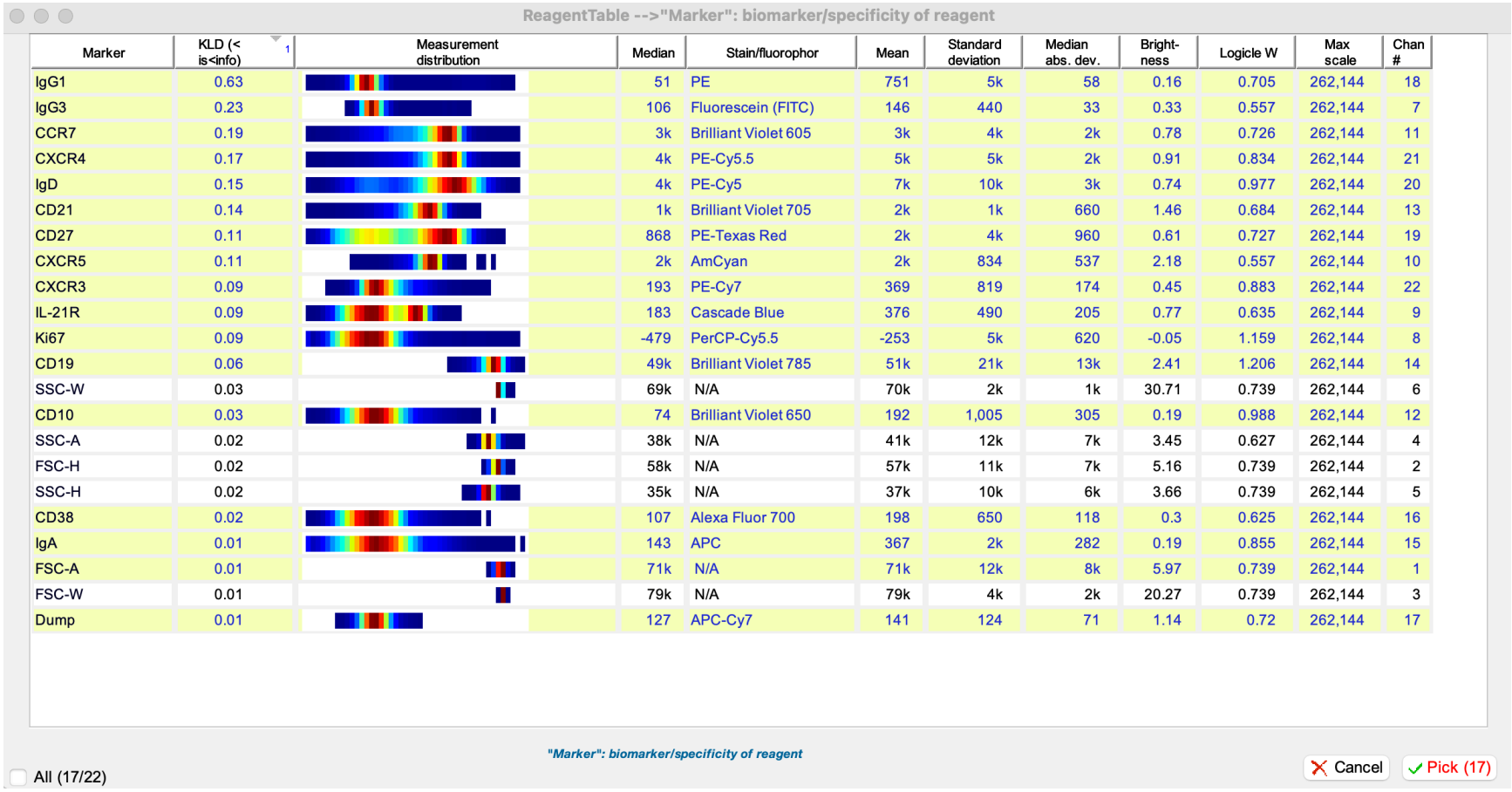
ReagentTable HC13, , 150416_HC13_037.fcs

Marker	KLD (< is<info)	Measurement distribution	Median	Stain/fluorophor	Mean	Standard deviation	Median abs. dev.	Bright-ness	Logicle W	Max scale	Chan #
IgG1	0.63		51	PE	751	5k	58	0.16	0.705	262,144	18
IgG3	0.23		106	Fluorescein (FITC)	146	440	33	0.33	0.557	262,144	7
CCR7	0.19		3k	Brilliant Violet 605	3k	4k	2k	0.78	0.726	262,144	11
CXCR4	0.17		4k	PE-Cy5.5	5k	5k	2k	0.91	0.834	262,144	21
IgD	0.15		4k	PE-Cy5	7k	10k	3k	0.74	0.977	262,144	20
CD21	0.14		1k	Brilliant Violet 705	2k	1k	660	1.46	0.684	262,144	13
CD27	0.11		868	PE-Texas Red	2k	4k	960	0.61	0.727	262,144	19
CXCR5	0.11		2k	AmCyan	2k	834	537	2.18	0.557	262,144	10
CXCR3	0.09		193	PE-Cy7	369	819	174	0.45	0.883	262,144	22
IL-21R	0.09		183	Cascade Blue	376	490	205	0.77	0.635	262,144	9
Ki67	0.09		-479	PerCP-Cy5.5	-253	5k	620	-0.05	1.159	262,144	8
CD19	0.06		49k	Brilliant Violet 785	51k	21k	13k	2.41	1.206	262,144	14
SSC-W	0.03		69k	N/A	70k	2k	1k	30.71	0.739	262,144	6
CD10	0.03		74	Brilliant Violet 650	192	1,005	305	0.19	0.988	262,144	12
SSC-A	0.02		38k	N/A	41k	12k	7k	3.45	0.627	262,144	4
FSC-H	0.02		58k	N/A	57k	11k	7k	5.16	0.739	262,144	2
SSC-H	0.02		35k	N/A	37k	10k	6k	3.66	0.739	262,144	5
CD38	0.02		107	Alexa Fluor 700	198	650	118	0.3	0.625	262,144	16
IgA	0.01		143	APC	367	2k	282	0.19	0.855	262,144	15
FSC-A	0.01		71k	N/A	71k	12k	8k	5.97	0.739	262,144	1
FSC-W	0.01		79k	N/A	79k	4k	2k	20.27	0.739	262,144	3
Dump	0.01		127	APC-Cy7	141	124	71	1.14	0.72	262,144	17

All (11/22)

Cancel Pick (11)

Add (mac key + select / Ctrl key + select) the 6 reagents with the highest KLD that are not already selected: BV605, PE-Cy5.5, AmCyan, PE=Cy7, Cascade Blue and PerCP+Cy5.5



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CCR7	0.19		3k	Brilliant Violet 605	3k	4k	2k	0.78	0.726	262,144	11
CXCR4	0.17		4k	PE-Cy5.5	5k	5k	2k	0.91	0.834	262,144	21
IgD	0.15		4k	PE-Cy5	7k	10k	3k	0.74	0.977	262,144	20
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CD27	0.11		868	PE-Texas Red	2k	4k	960	0.61	0.727	262,144	19
CXCR5	0.11		2k	AmCyan	2k	834	537	2.18	0.557	262,144	10
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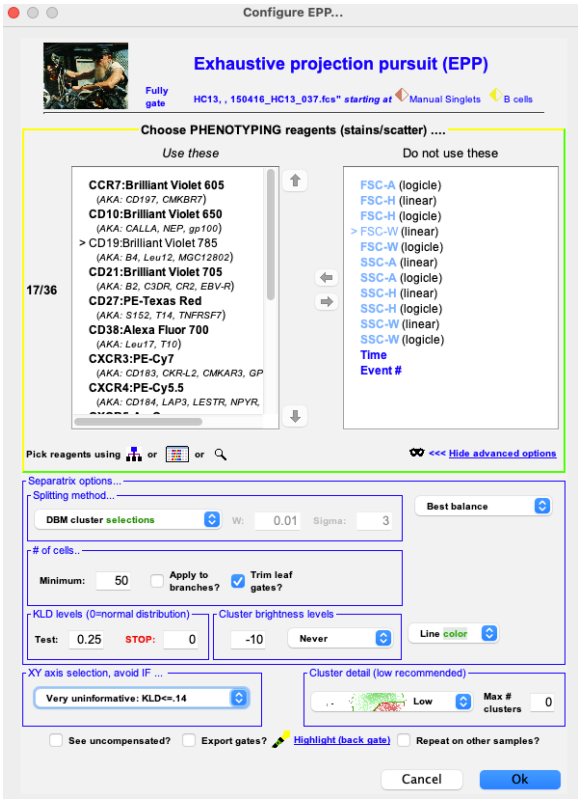
FSC-H	0.02		58k	N/A	57k	11k	7k	5.16	0.739	262,144	2
SSC-H	0.02		35k	N/A	37k	10k	6k	3.66	0.739	262,144	5
CD38	0.02		107	Alexa Fluor 700	198	650	118	0.3	0.625	262,144	16
IgA	0.01		143	APC	367	2k	282	0.19	0.855	262,144	15
FSC-A	0.01		71k	N/A	71k	12k	8k	5.97	0.739	262,144	1
FSC-W	0.01		79k	N/A	79k	4k	2k	20.27	0.739	262,144	3
Dump	0.01		127	APC-Cy7	141	124	71	1.14	0.72	262,144	17

6 Run EPP

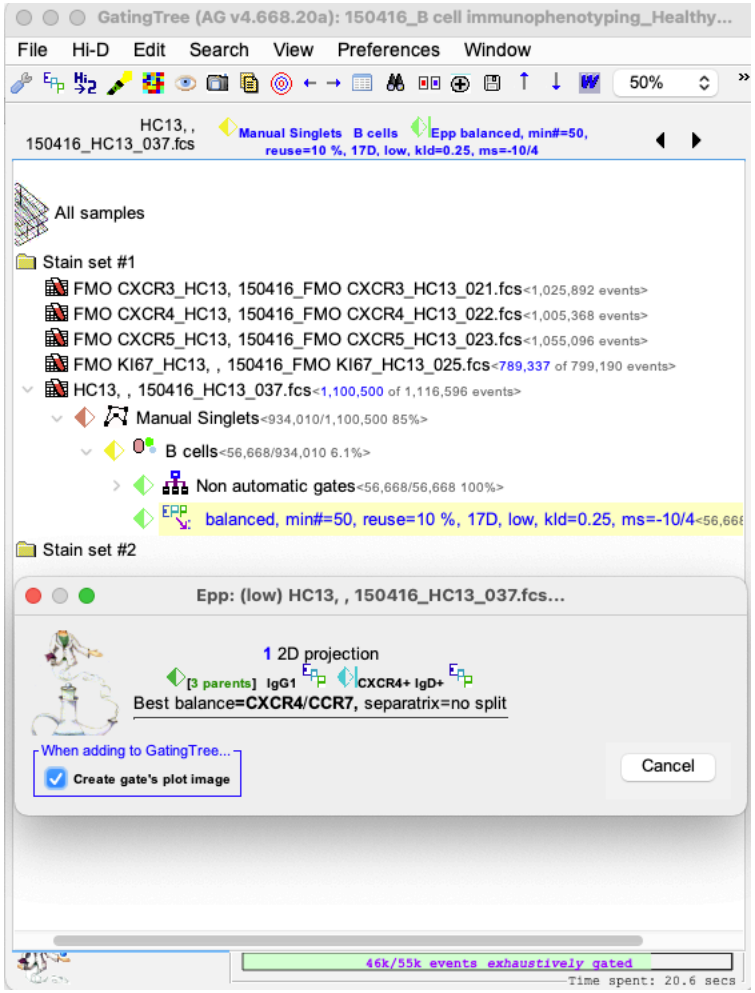
If your EPP configuration settings match the screenshot below then click the Ok button to run EPP!!



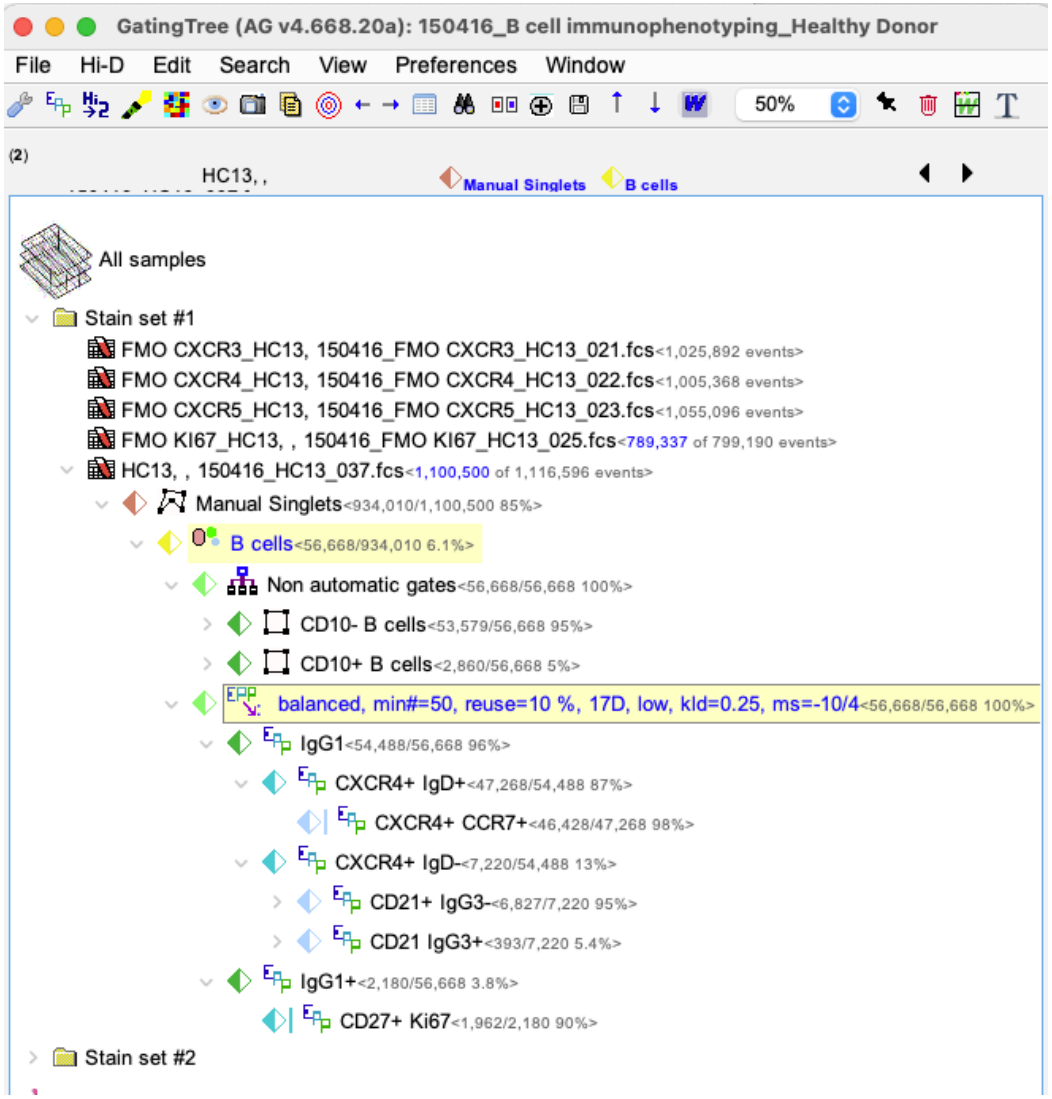
On a 6 core MacBook it takes EPP around 10 minutes to

1. Go through all X/Y pairings and find the top pairing where the data splits into two parts that have the best separation and are closest to each other's size
2. Narrow each of the two parts and repeat step 1 finding the next best data split to narrow into
3. Stop repeating steps 1 and 2 when good 2 way splits are no longer available

EPP in progress



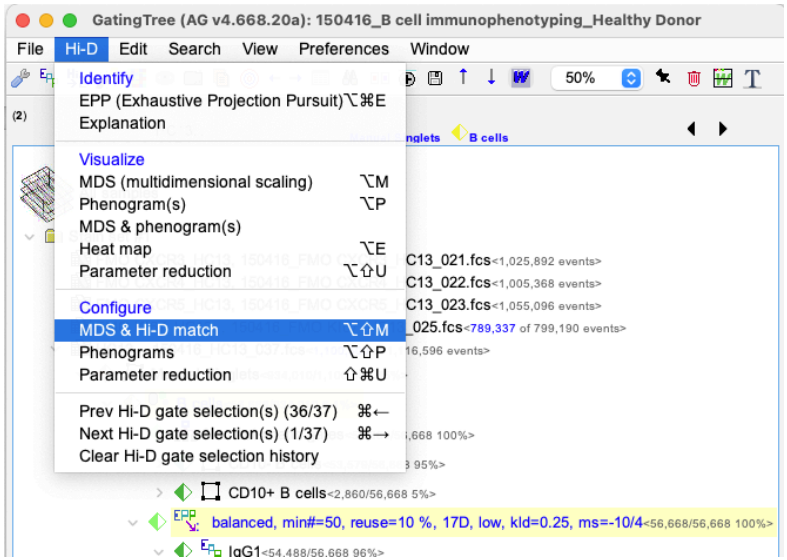
EPP completed



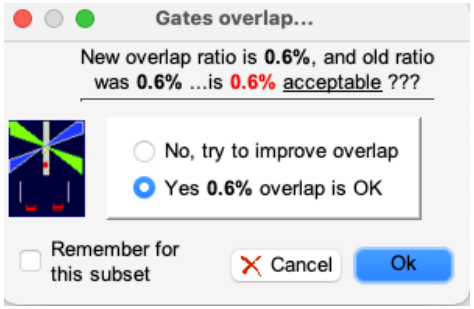
7 Compare EPP to OMIP investigator's manual gates

When EPP completes its run it then shows the menu for running AutoGate's matching between your fresh new EP gates and the OMIP investigator's manual gates for the same data

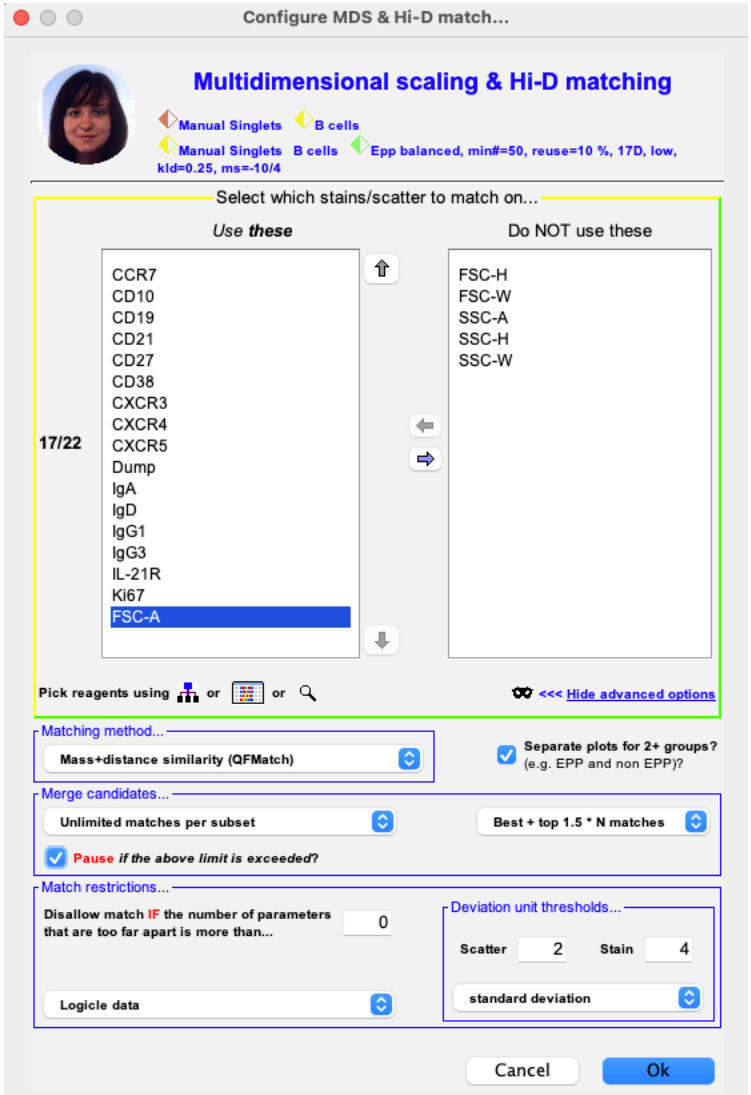
7.1 Select Configure MDS & HiD match from H->2 toolbar



7.2 Respond to overlap prompt

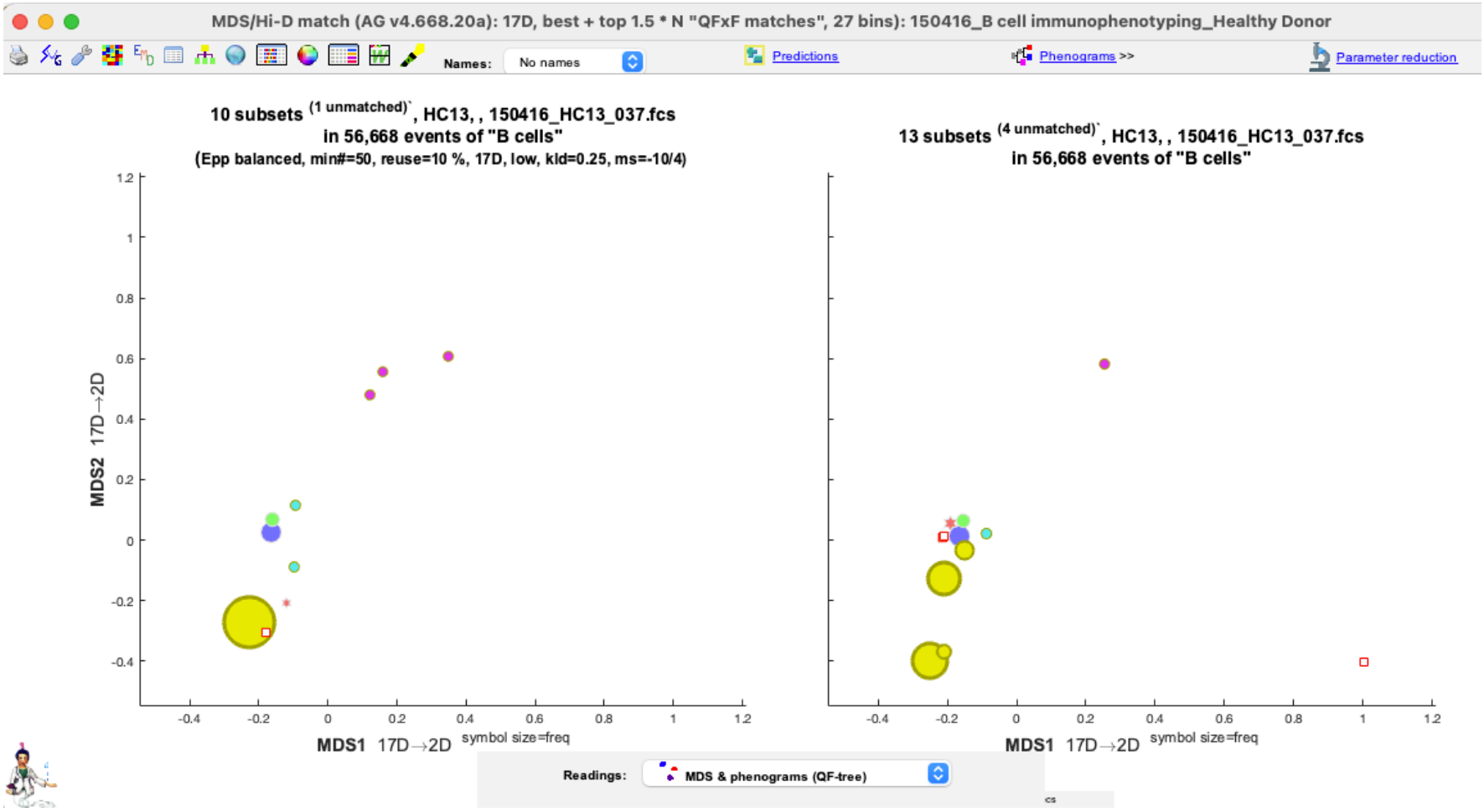


7.3 Ensure your settings match as below

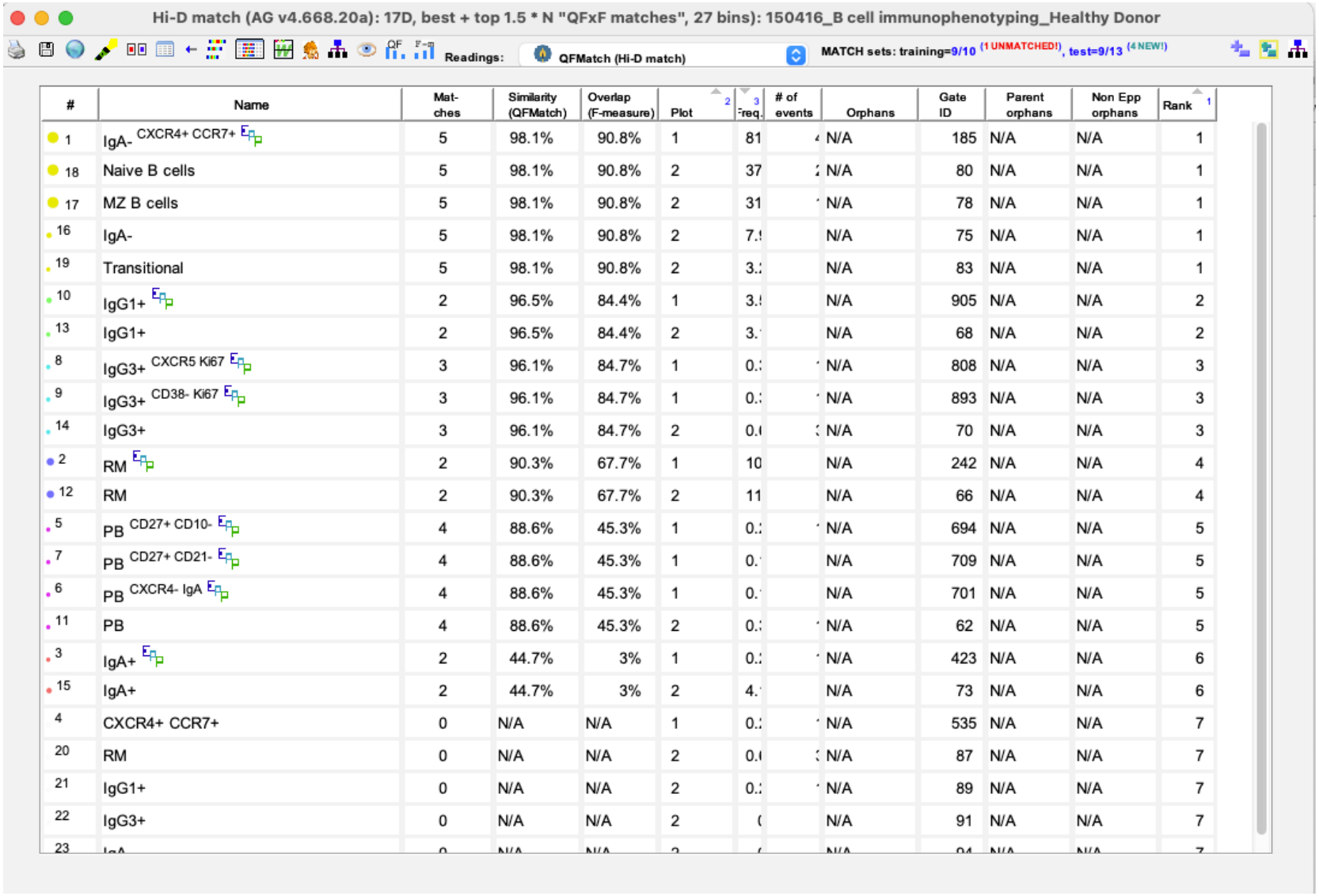


7.4 Evaluate matching

AutoGate shows you a resulting MDS (multi-dimensional scaling) display. This offers many features for comparing the EPP gates to the OMIP investigator's gates.



The quickest quantitative summary can be found by clicking on the table button of the toolbar which then shows best to least match with supporting statistics.



#	Name	Mat-ches	Similarity (QFMatch)	Overlap (F-measure)	Plot 2	Freq. 3	# of events	Orphans	Gate ID	Parent orphans	Non Epp orphans	Rank 1
1	IgA- CXCR4+ CCR7+	5	98.1%	90.8%	1	81		N/A	185	N/A	N/A	1
18	Naive B cells	5	98.1%	90.8%	2	37		N/A	80	N/A	N/A	1
17	MZ B cells	5	98.1%	90.8%	2	31		N/A	78	N/A	N/A	1
16	IgA-	5	98.1%	90.8%	2	7.!		N/A	75	N/A	N/A	1
19	Transitional	5	98.1%	90.8%	2	3.:		N/A	83	N/A	N/A	1
10	IgG1+	2	96.5%	84.4%	1	3.!		N/A	905	N/A	N/A	2
13	IgG1+	2	96.5%	84.4%	2	3.		N/A	68	N/A	N/A	2
8	IgG3+ CXCR5 Ki67	3	96.1%	84.7%	1	0.:		N/A	808	N/A	N/A	3
9	IgG3+ CD38- Ki67	3	96.1%	84.7%	1	0.:		N/A	893	N/A	N/A	3
14	IgG3+	3	96.1%	84.7%	2	0.(		N/A	70	N/A	N/A	3
2	RM	2	90.3%	67.7%	1	10		N/A	242	N/A	N/A	4
12	RM	2	90.3%	67.7%	2	11		N/A	66	N/A	N/A	4
5	PB CD27+ CD10-	4	88.6%	45.3%	1	0.:		N/A	694	N/A	N/A	5
7	PB CD27+ CD21-	4	88.6%	45.3%	1	0.		N/A	709	N/A	N/A	5
6	PB CXCR4- IgA	4	88.6%	45.3%	1	0.		N/A	701	N/A	N/A	5
11	PB	4	88.6%	45.3%	2	0.:		N/A	62	N/A	N/A	5
3	IgA+	2	44.7%	3%	1	0.:		N/A	423	N/A	N/A	6
15	IgA+	2	44.7%	3%	2	4.		N/A	73	N/A	N/A	6
4	CXCR4+ CCR7+	0	N/A	N/A	1	0.:		N/A	535	N/A	N/A	7
20	RM	0	N/A	N/A	2	0.(		N/A	87	N/A	N/A	7
21	IgG1+	0	N/A	N/A	2	0.:		N/A	89	N/A	N/A	7
22	IgG3+	0	N/A	N/A	2	(		N/A	91	N/A	N/A	7
23	IgA-	0	N/A	N/A	2	(		N/A	94	N/A	N/A	7

7.5 Mass + Distance similarity and overlap (F- measure)

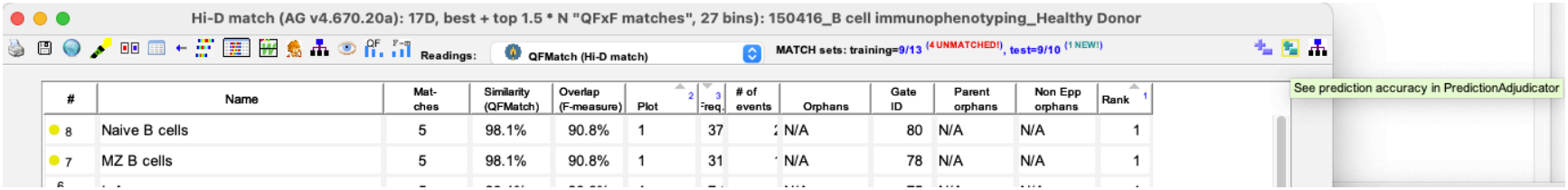
Click the highlighted buttons from the toolbar to view the graphs

#	Name	Mat-ches	Similarity (QFMatch)	Overlap (F-measure)	Plot	Freq.	# of events	Orphans	Gate ID	Parent orphans	Non Epp orphans	Rank
8	Naive B cells	5	98.1%	90.8%	1	37		N/A	80	N/A	N/A	1
7	MZ B cells			90.							N/A	1
6	IgA-			90.							N/A	1
9	Trar			90.							N/A	1
14	IgA-			90.							N/A	1
3	IgG			84.							N/A	2
23	IgG			84.							N/A	2
4	IgG			84.							N/A	3
21	IgG			84.							N/A	3
22	IgG			84.							N/A	3
2	RM			67.							N/A	4
15	RM			67.							N/A	4
1	PB	4	88.6%	45.3%	1	0.:		N/A	62	N/A	N/A	5

7.6 Prediction Adjudicator

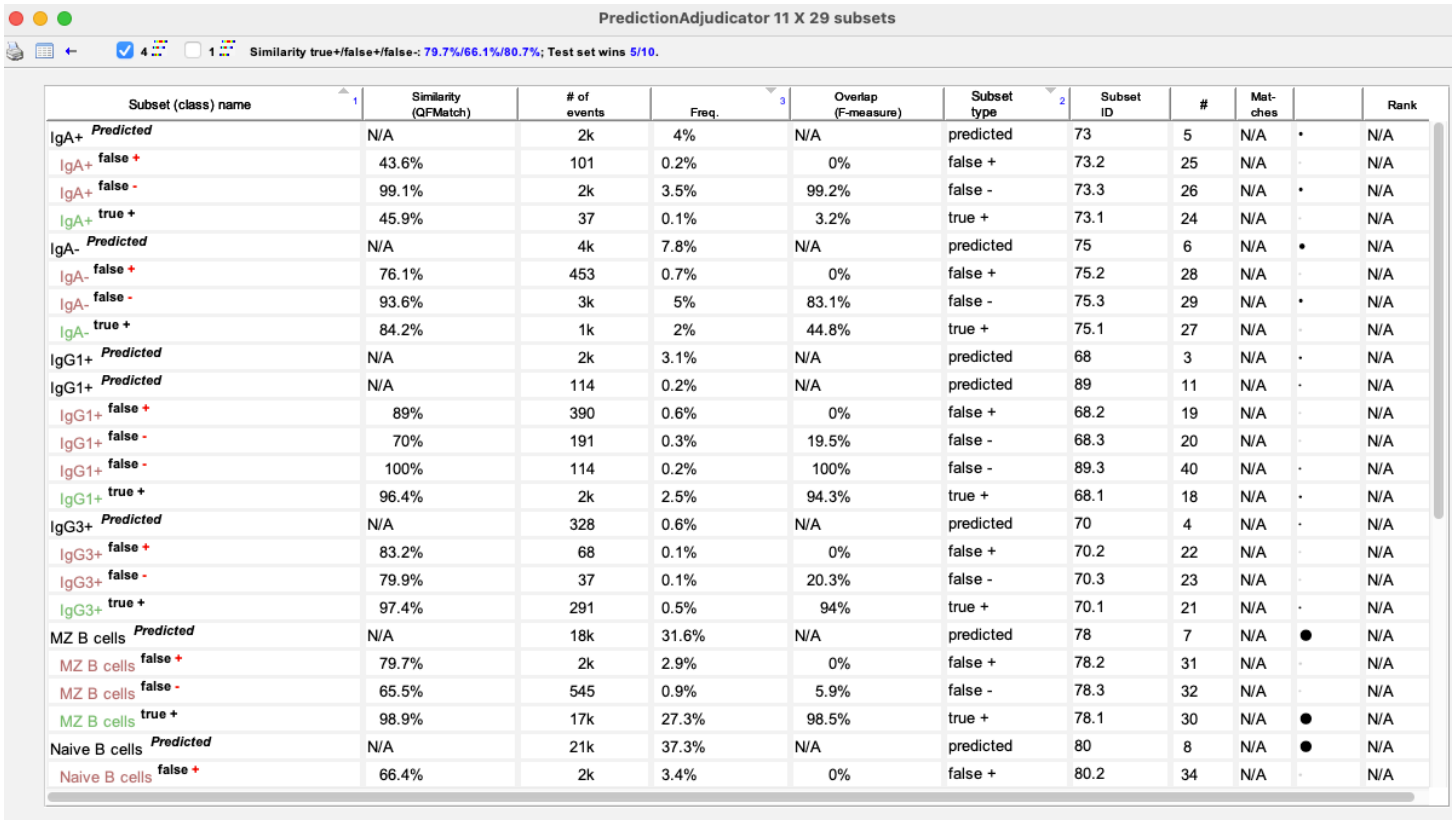
This helps determine how well one classification's subsets predict another's. PA reorganizes the predicting classifier's subsets into predicting subsets: true positive, false positive and false negative subsets. PA determines whether the false positives or false negatives have more QFMatch based similarity to the predicted subset.

Click the green +- button available in the top right corner of the above table



#	Name	Mat-ches	Similarity (QFMatch)	Overlap (F-measure)	Plot	Freq.	# of events	Orphans	Gate ID	Parent orphans	Non Epp orphans	Rank
8	Naive B cells	5	98.1%	90.8%	1	37		N/A	80	N/A	N/A	1
7	MZ B cells	5	98.1%	90.8%	1	31		N/A	78	N/A	N/A	1

PA results below



Subset (class) name	Similarity (QFMatch)	# of events	Freq.	Overlap (F-measure)	Subset type	Subset ID	#	Mat-ches		Rank
IgA+ *Predicted*	N/A	2k	4%	N/A	predicted	73	5	N/A	•	N/A
IgA+ false +	43.6%	101	0.2%	0%	false +	73.2	25	N/A		N/A
IgA+ false -	99.1%	2k	3.5%	99.2%	false -	73.3	26	N/A	•	N/A
IgA+ true +	45.9%	37	0.1%	3.2%	true +	73.1	24	N/A		N/A
IgA- *Predicted*	N/A	4k	7.8%	N/A	predicted	75	6	N/A	•	N/A
IgA- false +	76.1%	453	0.7%	0%	false +	75.2	28	N/A		N/A
IgA- false -	93.6%	3k	5%	83.1%	false -	75.3	29	N/A	•	N/A
IgA- true +	84.2%	1k	2%	44.8%	true +	75.1	27	N/A		N/A
IgG1+ *Predicted*	N/A	2k	3.1%	N/A	predicted	68	3	N/A	•	N/A
IgG1+ *Predicted*	N/A	114	0.2%	N/A	predicted	89	11	N/A	·	N/A
IgG1+ false +	89%	390	0.6%	0%	false +	68.2	19	N/A		N/A
IgG1+ false -	70%	191	0.3%	19.5%	false -	68.3	20	N/A		N/A
IgG1+ false -	100%	114	0.2%	100%	false -	89.3	40	N/A	·	N/A
IgG1+ true +	96.4%	2k	2.5%	94.3%	true +	68.1	18	N/A	•	N/A
IgG3+ *Predicted*	N/A	328	0.6%	N/A	predicted	70	4	N/A	·	N/A
IgG3+ false +	83.2%	68	0.1%	0%	false +	70.2	22	N/A		N/A
IgG3+ false -	79.9%	37	0.1%	20.3%	false -	70.3	23	N/A		N/A
IgG3+ true +	97.4%	291	0.5%	94%	true +	70.1	21	N/A	·	N/A
MZ B cells *Predicted*	N/A	18k	31.6%	N/A	predicted	78	7	N/A	●	N/A
MZ B cells false +	79.7%	2k	2.9%	0%	false +	78.2	31	N/A		N/A
MZ B cells false -	65.5%	545	0.9%	5.9%	false -	78.3	32	N/A		N/A
MZ B cells true +	98.9%	17k	27.3%	98.5%	true +	78.1	30	N/A	●	N/A
Naive B cells *Predicted*	N/A	21k	37.3%	N/A	predicted	80	8	N/A	●	N/A
Naive B cells false +	66.4%	2k	3.4%	0%	false +	80.2	34	N/A		N/A

7.7 Get False positive/negative rate

Click ‘See false positive/negative results’ blue button available on the top right corner of the toolbar