Flow Cytometry Research Group 2015 Study

Evaluating the Effects of Cell Sorting on Gene Expression

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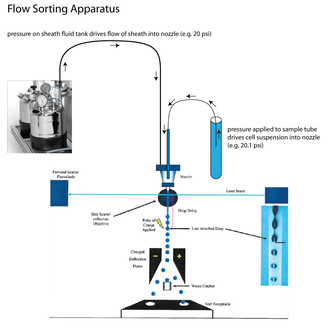
Introduction

The Flow Cytometry Research Group has continued with the goal to establish best practice guidelines for cell sorting conditions that minimize cell stress, perturbation, or injury to the sorted cell populations. In past FCRG studies, gene expression changes in sorted Jurkat cells, a human lymphoblastic T cell line, were correlated to nozzle size and sort pressure. The current study examined the effect sorting has on primary cells (C57Bl/6 mouse splenic B lymphocytes). B lymphocytes were isolated using multiple flow sorters under gentle (100 micron nozzle size/20 psi pressure) and stressful (70 micron nozzle size and 70 psi pressure) sort conditions. The sorts were performed using several instrument types to compare the differences in instrument designs (cuvette hybrid and jet-in-air) in addition to differences in sort conditions. Gene expression was assessed using Affymetrix Mouse Gene ST 2.0 microarrays using targets prepared from the NuGEN Pico reagents and Qiagen Micro minelute columns

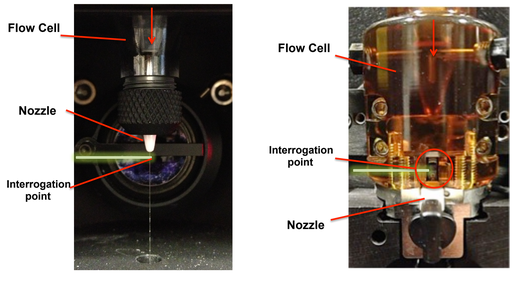
Background

When considering how to set up a cell sorter one of the significant variables that can have an effect on functional ability as well as cell health is the nozzle size and related pressure. A smaller nozzle requires a higher pressure be applied in order to generate a stable stream, with the opposite being true for a larger nozzle. A larger nozzle is thought to lead to a more gentle, but slower sort. This effect can be tested and is one of the goals of the current study.

There are also two common types of cell sorters, the Jet-in-air and Cuvette systems. The primary difference between the two systems is where the sample is excited. In the jet-in air system the sample stream is excited after it has passed out of a nozzle, whereas in the the cuvette system the excitation occurs while inside a quartz cuvette. Evidence has shown that this seemingly minor difference can lead to dramatic differences in cell health. Testing this effect is one of the ongoing goals of this research group.



Jet-In-Air ***Cuvette-hybrid***



2014-2015 Mouse B Cell Study

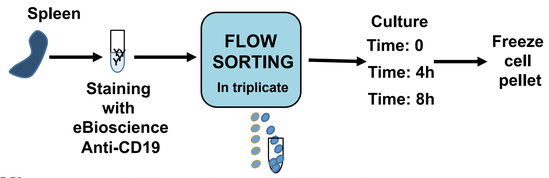
At 5 different sites (7 total instruments), primary cells from the spleen of a C57Bl/6 mouse were dissociated and CD19+ B cells were isolated via cell sorting. The B cells were evaluated after cell sorting by analyzing gene expression changes. RNA was generated from a selection of the sorted cells, amplified and analyzed via microarray.

➢**Sorters:** BD FACSAria II (4 sites) – Cuvette-hybrid system
BD Influx (1 site) – Jet-in-air system
BC MoFlo Astrios (2 sites) – Jet-in-air system

➢**Sort Conditions**: High Pressure – 70uM nozzle, 60-70psi
Low Pressure – 100uM nozzle, 20-25psi

➢**Culture Conditions:** 0, 4, and 8 hrs in culture post sort

Cell Sorting:



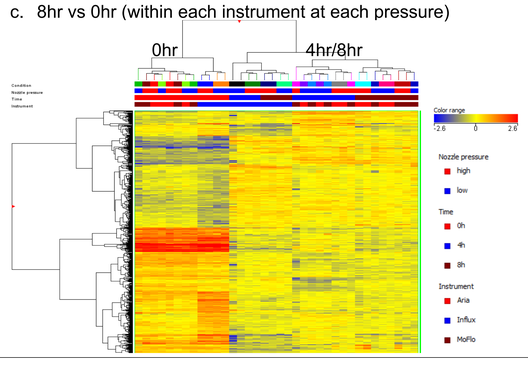
Microarray: 2 different sites and 3 different instruments

➢ 36 chips: 3 instruments, 3 time points, 2 conditions, 2 replicates

	FACSAria	Influx	MoFlo Astrios
Low Pressure	100micron/25 psi	100micron/20 psi	100micron/20 psi
High Pressure	70 micron/60 psi	70 micron/60 psi	70 micron/70 psi

Gene Expression Analysis Criteria:

1. Bottom 20th percentile probes across all samples were filtered out.
2. Remove any entities that had >25% CV
3. Differential expression using 2-way ANOVA
   a. Either between 4hr or 8hr as compared to the 0hr time point within each instrument and at both pressures
   b. Or between the different pressures at 0hr time points within each instrument
4. A 2-fold cutoff was applied to each comparison
5. Lists of differentially expressed entities were generated for the following comparisons.
   a. 0hr low vs 0hr high (within each instrument)
   b. 4hr vs 0hr (within each instrument at each pressure)
   c. 8hr vs 0hr (within each instrument at each pressure)



Pressure Induced Changes - Part 1

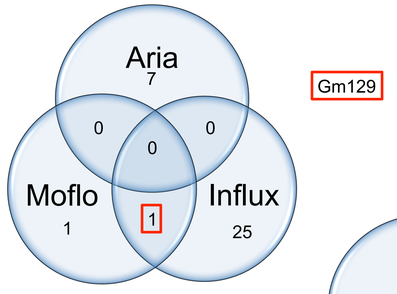
The analysis of these data was further focused on the gene expression variations between the "high" and "low" pressure conditions. To do this we took the fold change from 0hr to 4 or 8hr at low pressure conditions and compared that to the fold change from 0hr to 4 or 8hr at high pressure conditions.

Number of Genes/Group

Gene response	4 hour			8 hour		
	FACSAria	Influx	MoFlo Astrios	FACSAria	Influx	MoFlo Astrios
Up	7	26	2	7	7	3
Down	17	12	9	18	17	2
	Cuvette	Jet-in-Air		Cuvette	Jet-in-Air	

Genes "Up"

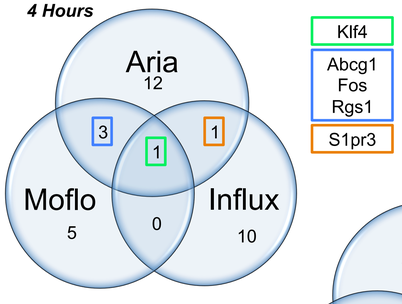
4 Hours



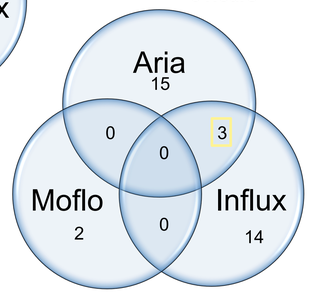
8 Hours

Genes "Down"

4 Hours



8 Hours



Pressure Induced Changes - Part 2

Genes Up	ARIA 4	ARIA 8	INFLUX 4	INFLUX 8	MoFlo 4	MoFlo 8
1700017B05Rik			x			
2310011J03Rik			x			
AF067061	x	x				
Ahr			x			
Ccl22	x	x				
Ccl3			x			
Clec12a					x	
Dusp10			x			
Egr1						x
Fam100a			x			
Fam46c			x	x		
Fosb						x
Gdap10			x			
Gla			x			
Gm12474						x
Gm129			x		x	
Gm17434			x			
Gm19450	x					
Gm19489				x		
Gm20022			x			
Gm2423			x			
Ifit1	x	x				
Il1r2			x			
Il2ra	x	x				
Lamp3			x			
LOC100862171				x		
Mir103-2			x	x		
Mir155|LOC100653389		x				
Nr4a1				x		
Oas1b	x					
Pde3b	x					
Per1			x			
Pik3r4			x			
Plaur			x			
Plf4			x			
Ppp1r15a			x			
Rgs1			x	x		
Slamf1		x				
Snora28			x			
Snord19			x			
St3gal6		x				
Trim34b|Trim34a			x	x		
Zc3h12c				x		
Grand Total	**7**	**7**	**26**	**7**	**2**	**3**

KLF4 is known to have a role in B cell proliferation and effects cyclin D and entry into S-Phase

S1pr3 is a G coupled receptor for sphingsine-1-phosphate and is a chemoattactrant and director of B cell trafficking

Genes Down	ARIA 4hr	ARIA 8hr	Influx 4hr	Influx 8hr	MoFlo 4hr	MoFlo 8hr
Jun						x
2610044O15Rik			x	x		
4931406C07Rik				x		
4932411G14Rik	x	x				
Abcg1	x				x	
Ahnak	x					
Anxa6	x					
Atf3				x		
Ccdc99				x		
Crisp3			x			
Cxcr4		x				
Dusp1			x			
Dusp10	x			x		
Dusp18			x			
Egr1					x	
Egr3					x	
Emp3					x	
Fam55b	x					
Fos	x	x			x	
Fosb			x			
Fyn		x				
Gm129	x					
Gm6377|Sh3bgrl		x				
Hes1			x			
Hmox1	x					
Id3				x		
Klf2	x	x				
Klf4	x	x	x	x	x	
Maf				x		
Mir27a		x			x	
Morf4l1|Gm6747				x		
Mtmfd2			x			
Mxi1		x				
Nr4a2		x				
Nr4a3		x				
Pcp4						x
Phxr1				x		
Plaur	x	x				
Plk2	x	x	x	x		
Rasd1	x			x		
Rgs1	x				x	
Rpp38			x			
S100a6		x				
S1pr3	x	x	x	x		
Slk1				x		
Sipa1l2		x				
Slamf1					x	
Trib1			x			
Vmn1LOC100862060	x					
Vps37b		x				
Zfp385a	x					
Zfp414		x				
Zfp948				x		
Zscan21				x		
Grand Total	**17**	**18**	**12**	**17**	**9**	**2**

Conclusions and Future Directions

Conclusions

- Cell sorting causes relatively few gene expression changes with a limited amount of overlap between instrument and time point.
- In agreement with past FCRG studies, although there were some alterations in gene expression, most of those changes had subsided with extended culture times.
- While gene expression changes were minor, cell viability was decreased after culture showing that cell sorting can have deleterious effects on cells (data not shown).
- Initial data (n=1) supports anecdotal evidence that sorting with the MoFlo Astrios has less effect on cells.

Future Directions

- These data represent a small portion of the total samples collected this year.
- Gene expression changes will be further explored using PCR with attention paid to differences between instrument types as well as continued exploration of the effects of pressure conditions.
- The FCRG plans to publish the results of this, and past years, studies.
- Please consider taking part in the FCRG survey (3 questions), regarding this project and future directions:

Acknowledgements

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