THE ADVANTAGES OF FLUORESCENT BARCODING OF HUMAN CELLS USING FLOW CYTOMETRY FOR HIGH THROUGHPUT TESTING WITH AN APPLICATION FOR HUMAN CLINICAL TRIALS

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ABSTRACT

Background: The use of fluorescent barcoding can be very advantageous in research for high throughput testing. Cell samples are stained with different concentrations of the reactive fluorescent dye which gives each sample a unique intensity distribution, which allow samples to be combined prior to antibody staining. The resolution of the staining profile is then recognized on the flow cytometer through data acquisition and analysis.

Methods: Experiments were conducted with human whole blood samples isolated, stimulated for pERK expression, fixed and frozen. Cells were barcoded with Violet Fluorescent Cell Barcoding Dye from Becton Dickinson and intracellular staining was performed for pERK. Cells were acquired on the BD FACSCanto™II flow cytometer.

Results: The individual profiles of 16 individual samples barcoded and stained with pERK and tested as one sample are highlighted in the results. Individual cell populations could be deconvoluted during analysis and the median fluorescence of the pERK was increased over background (unstimulated).

Conclusion: These results showed barcoding can be advantageous when a large number of samples are received. Advantages of this type of testing include minimize reagent consumption, lowering processing time and eliminating sample to sample or batch to batch variation when multiple samples can be processed in one single tube.

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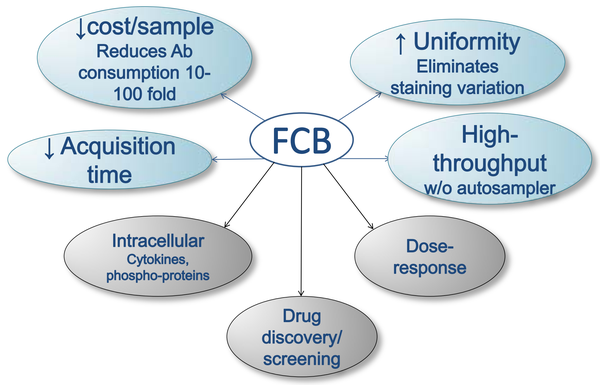
INTRODUCTION

Fluorescent cell barcoding (FCB) is a high throughput technique designed to multiplex samples prior to antibody staining and acquisition on the flow cytometer. Individual samples are barcoded with specific concentrations of fluorescent dyes before they are combined, stained and analyzed as a single sample. The unique fluorescent intensity distribution allows individual cell populations to be deconvoluted during analysis (Krutzik et al 2011).

ADVANTAGES [ ] & USES [ ]



The objective of this study was to perform FCB in 16 stimulated or unstimulated human samples for evaluation of intracellular expression of pERK, pS6 and p90RSK by flow cytometry.

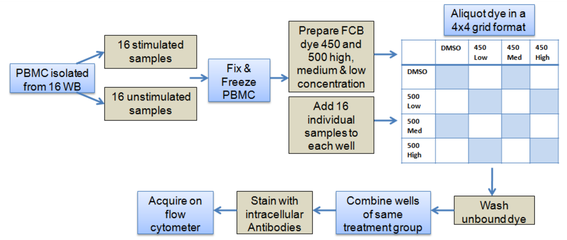
METHODS

Sample preparation: Fresh whole blood from 16 individual donors were obtained and PBMC was isolated. PBMCs were either stimulated with 10nM PMA or left unstimulated, then fixed and frozen. Samples were permeabilized using BD Phosflow™ Perm Buffer III.

Barcoding: Individual samples were added to the tubes containing various concentration of FCB Dye and incubated at 4°C for 25-30 minutes.

Staining & Flow Cytometry: Once cells were barcoded and washed, the 16 individual samples from each group were pooled together and stained with pERK-PE, pS6-AF488 and p90RSK-AF647. Single stained compensation controls were also prepared. After staining, the cells were washed and subjected to flow cytometry analysis performed by BD FACSCanto™ II equipped with BD FACSDiva™ 6.1.3 software and FlowJo vX.

OVERVIEW



RESULTS

Deconvoluted fluorescent barcoded samples

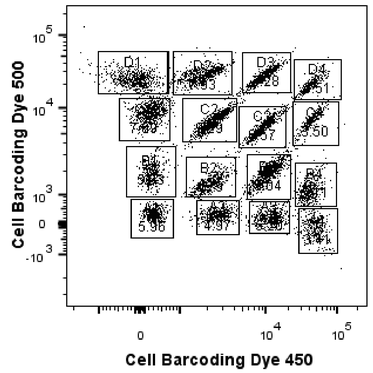


Figure 1. A representative cytogram of the 16 different samples that are deconvoluted after fluorescent barcoding with different concentrations of CBD450 and CBD500.

Multiple phopho-protein expression in stimulated human PBMC.

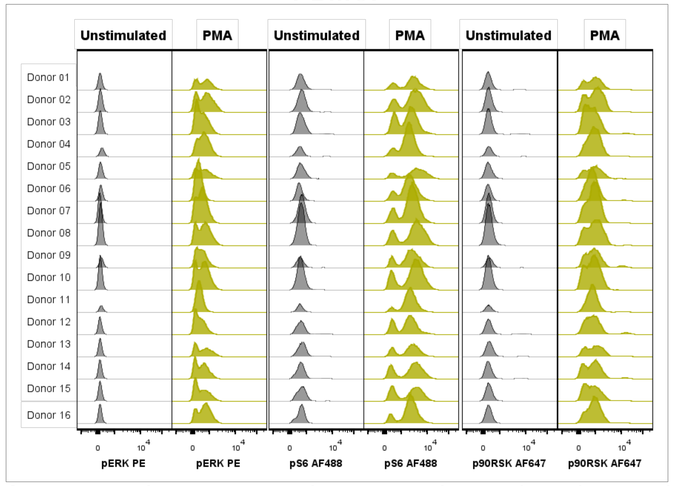
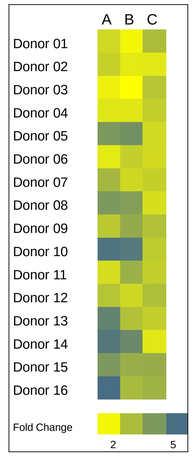


Figure 2. Histogram overlay to evaluate the change in expression of pERK, pS6 and p90RSK following stimulation with PMA. Deconvoluted samples were gated to evaluate the expression level in individual donor sample.

RESULTS (cont.)



Simplified analysis of high-throughput barcoding data as heat map to reveal protein expression change.

Figure 3. Fold change in expression of phosphorylated signaling proteins. Multiparametric profiling in 16 samples revealed an upregulation of pERK (**A**), pS6 (**B**) and p90RSK (**C**) following stimulation with PMA.

CONCLUSIONS

This study has shown that FCB can be successfully used as a high throughput flow cytometry technique without needing autosampler. This benefits the user in various ways, such as; uniformity in staining across all subjects, reduces antibody consumption, allows user to visualize data real time which is not possible with autosampler. FCB also enables multiparametric profilling of signaling proteins and data analysis can be performed with ease. Other studies have described the use of FCB on fresh whole blood samples. Here, we have shown that it is applicable in frozen PBMC samples and can be ideal for certain clinical trials.

REFERENCES

Krutzik, P.O, Clutter, M.R, Trjo, A, Nolan, G.P. 2011. Fluorescent Cell Barcoding for Multiple Flow Cytometry. Curr Protoc Cytom, Unit 6.31.

BD Biosciences. 2016. Technical Data Sheets. http://www.bdbiosciences.com/home.jsp

ACKNOWLEDGEMENTS

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