Cytometry Part A
Author Checklist: MIFlowCyt-Compliant Items

Requirement	Please Include Requested Information
1.1. Purpose	Optimization of Fluorescent Cell Barcoding technique for surface staining using human PBMCs
1.2. Keywords	flow cytometry, fluorescent cell barcoding, immunophenotyping, human PBMCs
1.3. Experiment variables	Barcoding dye working concentrations, number of cells required for combined samples, dye combinations
1.4. Organization name and address	National Heart, Lung, and Blood Institute, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892
1.5. Primary contact name and email address	Valentina Giudice: valentina.giudice@nih.gov
1.6. Date or time period of experiment	10/2016 to 01/2017
1.7. Conclusions	Using DyLight 350, DyLight 800, Pacific Orange, and CBD500, six, nine, or 36 human peripheral blood specimens can be efficiently barcoded and stained with a five-color phenotyping for TCR Vβ usage in CD4+ and CD8+ T cells, or alternatively with Aqua viability dye, for cell viability assessment.
1.8. Quality control measures	Controls were run for each conditions, as described in Material and Methods, Combination staining with Aqua Viability and FCB dyes paragraph and Combination staining with FCB dyes and antibodies paragraph. Dye stability was also tested at 24 and 48h.
2.1.1.1. (2.1.2.1., 2.1.3.1.) Sample description	human PBMCs, details in Material and Methods, Human samples.
2.1.1.2. Biological sample source description	Human peripheral blood mononuclear cells (PBMCs)
2.1.1.3. Biological sample source organism description	Human PBMCs were isolated from whole peripheral blood of healthy volunteers
2.1.2.2. Environmental sample location	-80°C storage
2.3. Sample treatment description	Detailed description in the following paragraphs in Material and Methods section: Staining with FCB dyes, Combination staining with Aqua Viability and FCB dyes, Combination staining with FCB dyes and antibodies
2.4. Fluorescence reagent(s) description	Material and Methods, Reagents
3.1. Instrument manufacturer	BD Biosciences. Details in Material and Methods, Data acquisition and analysis
3.2. Instrument model	LSR Fortessa cytometer. Details in Material and Methods, Data acquisition and analysis
3.3. Instrument configuration and settings	Details in Material and Methods, Data acquisition and analysis
4.1. List-mode data files	1) The link for peer-review process: https://flowrepository.org/id/RvFrqTKB41wdv33WH9vPM8DwLtkWUn2lFNJfopVhqw3fjwimWyOLZEgUvMxEKO4E 2) The repository identifier: http://flowrepository.org/id/FR-FCM-ZY3M
4.2. Compensation description	Compensation was performed using a bead standard for each fluorochrome and barcoded cells at the highest concentration of each dye for FCB dyes. Details in Material and Methods, Data acquisition and analysis
4.3. Data transformation details	All data were displayed with a bioexponential transformation, except for linear scaling (FSC and SSC). Histograms are shown using bioexponential transformation for the dye of interest (x axis) and normalized

	number of barcoded lymphocytes (y axis)
4.4.1. Gate description	Details in Material and Methods, Data acquisition and analysis; supplemental Figure 2; Results, co-staining with FCB dyes and antibodies. Flowjo file are also uploaded on repository
4.4.2. Gate statistics	Percentage of positive cells, MFI, CV, MFI fold change
4.4.3. Gate boundaries	

Notes

Feel free to use more space than allocated.

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