Detection of LacZ expression by FACS-Gal analysis

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Method Article

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Abstract

Introduction

Rosa26-LacZ transgenic reporter mice are a very common reporter line to determine Cre activity \(1). Since the disruption of target gene\(s) and the expression of the Rosa26-LacZ transgene are both controlled by the same Cre recombinase, LacZ expression/activity can serve as a useful marker of the cells with targeted gene disruption. X-gal staining is a common assay used to detect LacZ+ cells in many tissues. In 1988, Nolan, et. al. \(2) developed FACS-Gal analysis, a FACS-based detection of LacZ+ cells with a fluorogenic substrate, FDG, of galactosidase \(LacZ enzyme). Galactosidase cleaves FDG and releases a fluorescence product FITC \(3). However, it is common that LacZ signals varies from sample to sample. Here we establish a procedure to ensure reliable and consistent results for the whole hematopoietic system, with the exception of terminally differentiated erythroid cells, or red blood cells that express much lower LacZ proteins.

Reagents

1. 70µm cell strainer \(Fisher, Cat. 352350) 2. Amber eppendorf tubes \(USA scientific) 3. 20mM FDG stock solution \(Fluorescein di\[β-D-galactopyranoside], Sigma, F2756) Dissolve FDG in DMSO and ethanol solution \(DMSO:ethanol=1:1) completely, and then gradually add ice-cold water \(DMSO:ethanol:water=1:1:8) into FDG drop by drop. Aliquot and keep the FDG solution at -20ºC for long-term storage. Make FDG diluent \(DMSO:ethanol:water=1:1:8) as FDG control stock solution. Dilute 1:10 to make a working FDG solution or FDG control solution when needed. 4. HBSS+ buffer, ice-cold \(1x HBSS, 2% Fetal calf serum, 10mM Hepes buffer \[pH 7.2], 1% Penicillin/streptomycin) 5. Bone marrow harvesting medium, ice-cold \(1x DMEM, 2% Fetal calf serum, 10mM Hepes buffer \[pH 7.2], 1% Penicillin/streptomycin) 6. Fluorescence-conjugated antibodies \(BD Pharmingen, eBiosciences, or Biolegend) 7. 7-AAD solution \(BD Pharmingen)

Equipment

Water bath at 37ºC BD FACScan, Calibur, Canto or LSR analyzer

Procedure

1. Prepare single cell suspension. a) Harvest bone marrow cells by flushing femur with ice-cold bone marrow harvesting medium. b) Filter the suspended cells through 70µm cell strainer while keeping cells on ice. c) Count the cell concentration. d) Centrifuge the filtered cells at 1500rpm for 5-10 min. e) Resuspend the cells with HBSS+ staining buffer \(HBSS+, ~2×10^7 cells/mL if possible). 2. Load FDG into cells. a) Aliquot 100µl suspended cells for staining. Prepare controls including unstained control, single color controls for FACS compensation. b) Prepare 2mM FDG working solution by diluting 20mM FDG stock solution with ice-cold distilled water. Prepare working FDG control solution by diluting FDG

control stock solution by 1:10. Aliquote 100µL FDG working solution or control solution to amber tubes. c) Prepare 15mL tubes with 2mL HBSS+ buffer and keep on ice. d) Make sure all the sets of cell sample tubes, FDG tubes and 2mL HBSS+ tubes are ready for the following steps. e) Prewarm cell sample tubes and corresponding FDG \(or control) tubes in 37ºC water bath for 10min. 2mL HBSS+ tubes are kept on ice during loading. f) Follow the FDG loading strategy shown in Figure 1 to load FDG into cells. Transfer prewarmed cells into corresponding FDG tubes. Mix thoroughly. Return to 37ºC bath for exactly 1 min. Stop the FDG loading at the end of one min by transferring mixture into 2mL ice-cold HBSS+. g) Keep on ice for 1.5 hours to allow accumulation of FITC release from FDG in LacZ+ cells. h) Centrifuge at 1500rpm for 7 minutes, discard supernatant and resuspend cell pellets with 100µL ice-cold HBSS+ buffer. 3. Stain cells with antibodies. a) Add 2µl antibodies to cells and slowly rock at 4ºC for 15 min. b) Centrifuge at 1500rpm for 5-10 min, discard supernatants and resuspend cell pellets with 200ul ice-cold HBSS+. c) Add 7µl 7-AAD solution to 200µl cells 4. Perform FACS analysis. a) Adjust voltage. b) Run compensation. c) Run samples and collect FACS data. d) Analyze your FACS data.

Timing

It takes 6 hours to perform the experiment.

Critical Steps

Step 1.e. Take care to remove any remaining harvesting medium or buffer in the tube. Single cell suspension has to be made in HBSS+ buffer, which has been tested to work best for FDG loading. Step 2.c-e. To make sure to incubate cell and FDG mixtures for exactly 1 minute, the FDG loading strategy in Figure 1 should be used. Step 2e. From this step downward, all samples needs to be kept on ice at all.

Troubleshooting

1. Dim LacZ+ cell signal. There are three possible issues: a) Make sure that the volume ratio of cell to FDG is exactly 1:1. b) Make sure to follow the FDG loading strategy shown in Figure 1. c) The cells have to be kept on ice after FDG loading. Each step should be performed on ice. Even when the FDG-loaded cells are carried around, they have to be on ice.

Anticipated Results

1. LacZ+ cells show much higher FITC signaling on FACS plots than LacZ- cells \(2).

References

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Figures

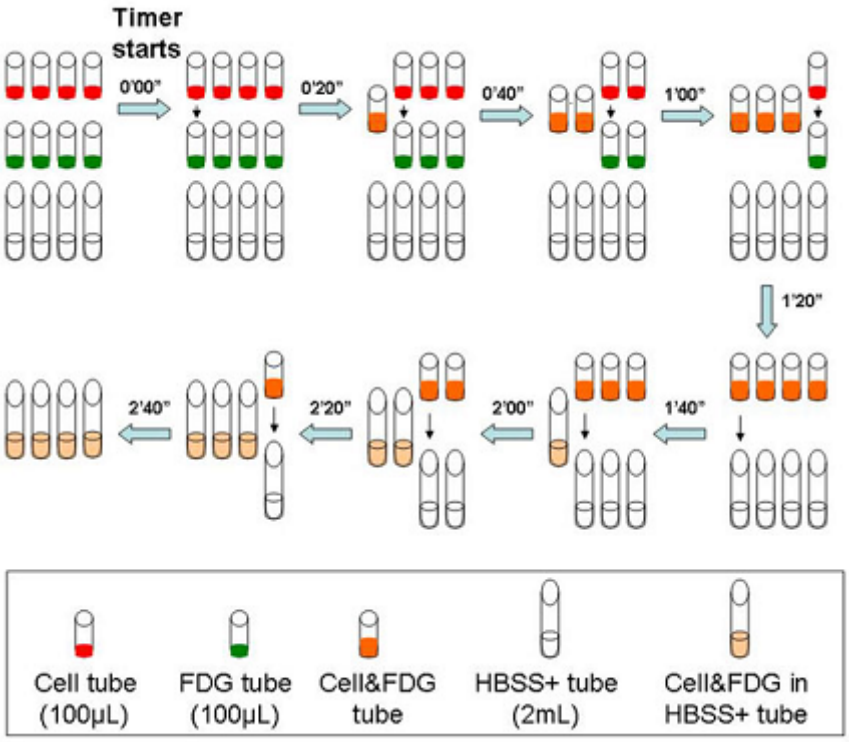

Figure 1

FDG loading strategy 1. Make sure all tubes (FDG/control and 2mL HBSS+) are properly prepared before loading. Note that the 2mL HBSS+ tubes are kept on ice during loading. 2. Place paired tubes of FDG/Control and cells (maximum of four pairs in each group) simultaneously into a 37 C water bath at 3-minute intervals. 3. After 10 minute of warming, load FDG into cells as shown in the figure below: a) Set timer at 0'00" and start counting up. Take the first paired tube of cells and transfer cells into the corresponding FDG tube. Mix well. Place the FDG and cell mixture tube back into the 37 C water bath exactly at each 20 second mark. b) Transfer the mixtures into the corresponding 2mL HBSS+ (on ice) exactly at the end of each tube's 1 minute incubation time. 4. After finishing the group, the next group will be ready for mixing and incubation in 20 seconds. When the next group is ready, repeat loading steps 2a,b. A larger version of this figure can be found "here":http://protocols.nature.com/image/show/997