LacZ FACS analysis (FDG)

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Summary

One of the most common reporter genes used in molecular biology applications is the E. coli lacZ gene that codes for an active subunit of β-galactosidase in vivo. Although chromogenic assays of β-galactosidase activity (i.e. X-gal) are useful, the application of the fluorescent substrate fluorescein di-D-β-galactopyranoside (FDG) has been shown to be several orders of magnitude more sensitive. Because of this high sensitivity, use of FDG allows quantitation of LacZ expression in single, viable eukaryotic cells, whereas other assays often result in dead cells.
Note: Alternative fluorescent substrate 3-carboxhyumbelliferyl-β-galactopyranoside (CUG) is available but need a UV laser to detect (Zeng and Nusse, 2010).
This protocol is a modified version of the Nature protocol 446.
http://www.nature.com/protocolexchange/protocols/446

Reagents

1. 1 mM FDG stock solution
   Dissolve FDG (M0250, Marker Gene) in DMSO/EtOH (1:1) solution completely, and then gradually add ice-cold water into FDG drop by drop (DMSO:EtOH:H_2O=1:1:8). Aliquot and keep the FDG solution at -20 ºC for long-term storage. Make FDG dilutent (DMSO:EtOH:H_2O=1:1:8) as FDG control stock solution.
2. ice-cold HBSS+ buffer (1xHBSS [home made], 2%FBS, 10mM Hepes [pH7.2, BP310-500, Fisher])
3. ice-cold culture medium without FBS

Procedure

1. Prepare single cell suspension. Centrifuge, and aspirate the supernatant.
2. Prepare 0.2 mM FDG working solution by diluting 10 mM FDG stock solution with **ice-cold** medium (no serum). Prepare working FDG control solution by diluting FDG control stock solution as well.
3. Prepare 5-mL FACS tubes with 2-mL HBSS+ buffer and keep on ice.
4. Make sure all the sets of cell sample tubes, FDG tubes and HBSS+ tubes are ready for the following step. (an example of FDG loading strategy is shown in Fig1)
5, Prewarm cell sample tubes and corresponding FDG (or control) tubes in 37ºC water bath for 10 min. 2-mL HBSS+ tubes are kept on ice during loading.
6. Load FDG into cells. Transfer prewarmed FDG working solutions into cell pellets. Mix thoroughly. Return to 37ºC bath for exactly 1 min. Stop the FDG loading at the end of 1 min by transferring mixture into 2-mL ice-cold HBSS+.
7. Keep on ice for 1.5 h to allow accumulation of FITC release from FDG in LacZ+ cells.
8. Centrifuge at 1500 rpm for 5 min.

9. Perform antibody staining if necessary.
10. Run samples on FACS

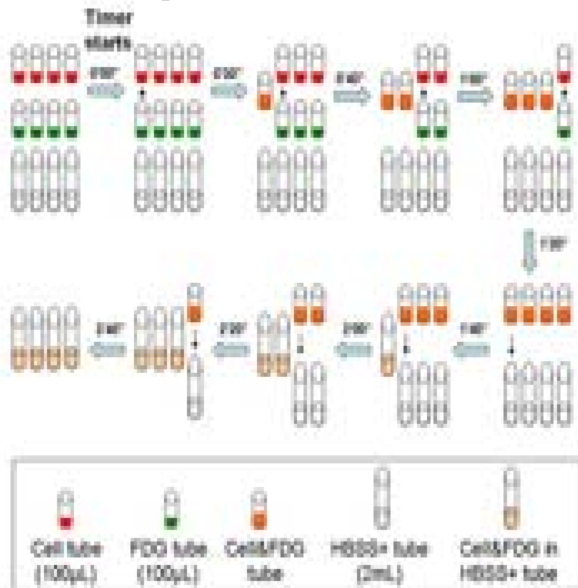

Fig1 FDG loading flowchart

Notes

I initially tried the Nusse group protocol with 2 mM FDG (CUG) for 45 min incubation at 37ºC (Zeng and Nusse, 2010), but the background was extremely high. I was able to minimize the background with 0.2 mM FDG for 1-min FDG loading and 90-min FITC release and was still able to detect signals. Please note that the assay was optimized for detection of LacZ positive cells in Axin2$^{LacZ/+}$ mammary gland.

References

Zeng, Y.A., and Nusse, R. (2010). Wnt proteins are self-renewal factors for mammary stem cells and promote their long-term expansion in culture. Cell stem cell *6*, 568-577.