MoFlo™ Fluorescence Resonance Energy Transfer

Introduction

Fluorescent proteins have become a mainstay in the study of protein localization, protein-protein interaction and gene expression. By rendering a protein of interest natively fluorescent, fluorescent proteins allow the intracellular localization and quantitation of that protein without invasive labeling procedures. Multicolor applications for fluorescent proteins include fluorescent protein-paired fluorescence resonance energy transfer.[1-3]

Fluorescence resonance energy transfer, or FRET, provides the means for examining the proximity of molecules within the range of 10 to 100 angstroms. In FRET studies (Figure 1), a donor fluorophore is optimally excited and, if a suitably chosen acceptor fluorophore is within the specified distance, the donor fluorescence emission excites the acceptor fluorophore and the acceptor fluorophore fluorescence is seen. If FRET occurs, donor fluorescence should decrease in intensity, while acceptor fluorescence should increase. When FRET is used in conjunction with the MoFlo high performance cell sorter, researchers can acquire information about molecular proximity at rates exceeding 100,000 data points/second. They also can collect cells of interest at rates approaching 70,000 cells/second.[4]

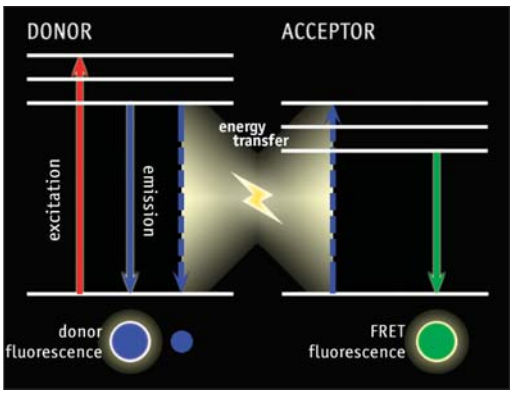


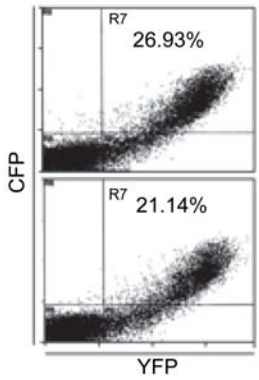
Figure 1

Materials and Methods

In order to perform a FRET experiment optimally using flow cytometry, several control samples must be included in the experimental protocol. The following suggested protocol illustrates samples required to perform a CFP/YFP FRET analysis. Please note that optimally all samples should be of the same cell type, and single transfectants should use the same fusion proteins as the experimental sample.

1. A non-fluorescent protein transfectant. This cell sample is used for initial instrument setup, establishing photo-multiplier tube (PMT) settings and determining a live-cell gate using light scatter.
2. A CFP-only transfectant. This sample is used to fine tune PMT settings and to monitor spectral bleed-over into the YFP and FRET detectors.
3. A YFP-only transfectant. This sample is again used to fine tune PMT settings and to monitor spectral bleed-over into the CFP and FRET detectors.
4. A CFP and YFP dual transfectant, where the two proteins are known not to interact. Here dual CFP/YFP expression should be observed without any increase in FRET.
5. The actual CFP/YFP experimental sample. FRET can now be reliably detected if present.

In the experiment shown here, FRET provides information about protein interactions implicated in the pathogenesis of Alzheimer's disease. CFP and YFP fusion proteins were made in pGEX vectors and mutations were introduced using the transformer site-directed mutagenesis kit. Human embryonic kidney (HEK) 293T cells were plated and co-transfected with the fusion proteins using Fugene 6. Cells were harvested 18-24 hours after transfection in their conditioned media and strained through nylon mesh to separate the cells. Cells were analyzed on a MoFlo equipped with 488 nm argon (75 mW) and 413 nm krypton (60 mW) lasers. For YFP detection, 530/40 bandpass filter and 1.0 neutral density filter were used with a log amplification of 400-450 volts. For CFP detection, 473/12 bandpass filter and 0.3 neutral density filter were used with a log amplification of 470-550 volts. FRET detection was carried out with 550/30 bandpass filter and



ARH interacts with AbPP in vivo. These plots illustrate co-transfection of YFP and CFP fusion proteins. YFP-AID (Y-AID), CFP-ARH (C-ARH) and CFP-ARHΔPTB (C-ARHΔPTB) are fusion proteins expressed in vivo to demonstrate the interaction between the AID portion of the amyloid protein precursor (AbPP) with the autosomal recessive hypercholesterolemia (ARH) adapter protein.

Figure 2a

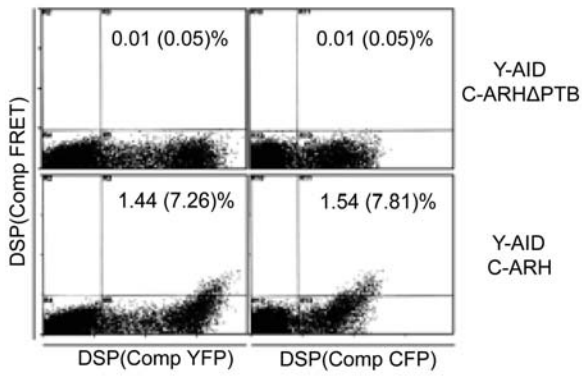


Figure 2b

An increase in the intensity of cells on the Y axis indicates FRET between Y-AID and C-ARH (lower plots), but not between Y-AID and C-ARHΔPTB (upper plots). Cells in the upper right quadrant in plots in Figure 2b were scored as being positive for FRET, and percentages in parentheses are the fraction of co-transfected cells (R7 in Figure 2a), which show FRET.

510 dichroic longpass filter at a log amplification of 475 volts. Acquisition and analysis were done using Summit software.

Results

Cells transfected with a CFP fusion protein or a YFP fusion protein alone exhibit no FRET. Additionally, cells co-transfected with the fusion proteins CFP-ARHΔPTB and YFP-AID also do not demonstrate FRET (Figure 2b, upper plots). However, cells co-transfected with the fusion proteins CFPΔARH and YFP-AID are positive for FRET, as indicated by the distinct shift in fluorescence emissions (Figure 2b, lower plots). This shift indicates the proximity of these two proteins during Alzheimer's pathogenesis and demonstrates the role of the MoFlo in such molecular interaction studies.[5-6]

Discussion

In the rapidly evolving proteomics era, applications for fluorescent proteins, such as CFP, YFP, GFP and BFP, continue to grow. Whether these proteins are simple indicators of gene expression levels or tools in fluorescence resonance energy transfer studies, the MoFlo High Performance Cell Sorter is an ideal platform for detecting these proteins and isolating cells with desired expression patterns.

References

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