

"Go with the Flow..."

Cytometry Research, LLC

"...Flow Cytometry, that is!"

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Cutting Edge Technology for Cutting Edge Scientists

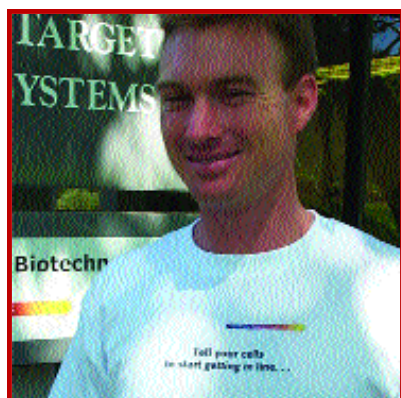
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Simultaneous Detection of Bacteria Expressing GFP and DsRed Genes with a Flow Cytometer

Mikael Maksimow, Kaisa Hakkila, Matti Karp, and Marko Virta (2002) *Cytometry* 47:243-247.

Using flow cytometry, researchers took *Escherichia coli* cells producing red fluorescent protein of *Discosoma sp.* (drFP583 DsRed) and enhanced green fluorescent protein (GFP) to study whether GFP and DsRed could be detected simultaneously from a single bacterial cell. Although GFP has been commonly used in flow cytometry as a reporter protein for monitoring gene expression in both eukaryotic and bacterial cells, until now there did not exist another fluorescent protein with an emission peak beyond 529 nm that could be used simultaneously with GFP. In this study, researchers used plasmids pDsRed and pEGFP to construct new plasmids that encode tandem fusions of enhanced GFP and DsRed. The production of these two fusion proteins in *E. coli* MC1061 cells resulted in bacteria that simultaneously emitted equivalent levels of both green and red fluorescence and could easily be detected using a 488 nm single laser excitation light source.

Instrument set-up consisted of collecting green fluorescence emission with a 525/10-nm (FL1) band pass filter and red fluorescence through a 620/10-nm (FL3) band pass filter. The slight spectral overlap was adjusted with a minor compensation of 6% (FL1-FL3). It was noted that cultivation of bacteria at 20°C gave the most reproducible results. Negative controls for red and green fluorescence were set at less than 2%. Analysis results demonstrated that 90% of the cells having the EGFP gene exhibited green fluorescence and about 85% of the cells having the DsRed gene were positive for red fluorescence. Accordingly, 85% of those cells containing one of the fusion proteins were positive for red and green fluorescence. The results clearly demonstrate that it is possible to simultaneously detect bacteria expressing GFP and DsRed genes using flow cytometry.



Just in time for summer, Cytometry Research releases its first T-shirt! The front says, "Tell your cells to start getting in line..." The back says, "The FLOW doctor is in!"



That's right! The FLOW doctor is in, so give us a call to schedule your next FACS analysis or cell sorting appointment. And, if you want one of these HOT T-shirts for your own, just solve the puzzle on the back cover of this issue and fax it in to Cytometry Research.

HOT FACES TECHNOLOGY

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Fun "FACS**"

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