evrogen

High effective FRET pairs

Ranging in color from blue to far-red, Evrogen fluorescent proteins can be used in fluorescence resonance energy transfer (FRET) applications as donors and acceptors of fluorescence. TagFPs are perfect for *in vivo* protein interaction studies by FRET due to their improved performance in fusions. TagBFP-TagGFP2 and TagGFP2-TagRFP pairs show the highest FRET efficiency among the tested TagFPs combinations and compare favorably to other available FRET pairs of monomeric fluorescent proteins.

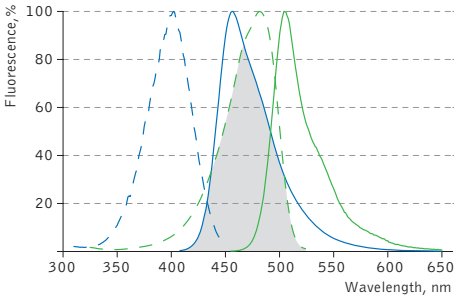
Characteristics	TagBFP-TagGFP2	TagGFP2-TagRFP
FRET efficiency (E)	0.57	0.50
Calc. Forster distance (R_0)	5.25	5.70
FRET donor	TagBFP	TagGFP2
Fluorescence color	blue	green
Excitation maximum, nm	402	483
Emission maximum, nm	457	506
Brightness, % of EGFP	99	105
pKa	2.7	5.0
FRET acceptor	TagGFP2	TagRFP
Fluorescence color	green	red
Excitation maximum, nm	483	555
Emission maximum, nm	506	584
Brightness, % of EGFP	105	148
pKa	5.0	3.8

TagBFP-TagGFP2 pair

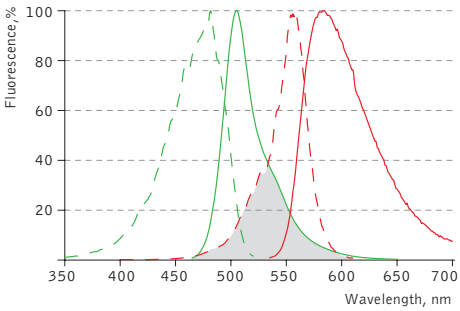
The calculated Forster distance (R_0 = 5.25 nm) for TagBFP-TagGFP2 pair is larger than those reported for the standard ECFP-EYFP and mCyPet-mYPet pairs (R_0 = 4.86 nm and 4.93 nm, respectively). Calculation of FRET efficiency (E) based on the increase of donor emission upon cleavage of the fusion protein in vitro produced E=0.57 for TagBFP-TagGFP2. For comparison, E=0.38 for EBFP2-TagGFP2 pair; E=0.42 for ECFP-EYFP pair; and E=0.51 for mCyPet-mYPet pair.

When TagBFP and TagGFP2 free proteins are coexpressed in HeLa cells, the cross-bleed-corrected FRET normalized to fluorescence of donor is 0.85%. Under the same experimental conditions, the cross-bleed-corrected FRET between ECFP and EYFP free proteins coexpressed in HeLa cells is 6.2%, confirming their weak dimerization. In other words, TagBFP and TagGFP2 proteins derived from the different marine sources and, as a result, lacking ability to form heterodimers, provide more than 6-fold lower background for FRET analysis than the weakly dimerizing FRET pairs, such as the ECFP-EYFP.

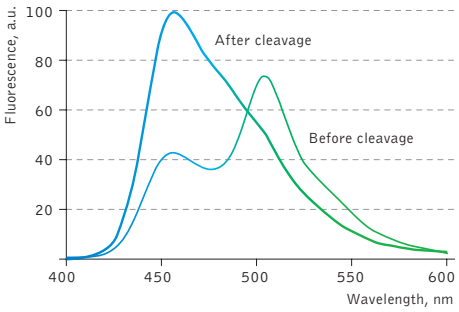
Thus, TagBFP-TagGFP2 pair is not only superior to other BFP-GFP pairs, but is one of the best among available FRET pairs of the true monomeric fluorescent proteins [Subach *et al.*, 2008].



Excitation (dashed lines) and emission (solid lines) spectra of TagBFP (blue) and TagGFP2 (green) are shown individually. Spectral overlap is filled with grey.



Excitation (dashed lines) and emission (solid lines) spectra of TagGFP (green) and TagRFP (red) are shown individually. Spectral overlap is filled with grey.



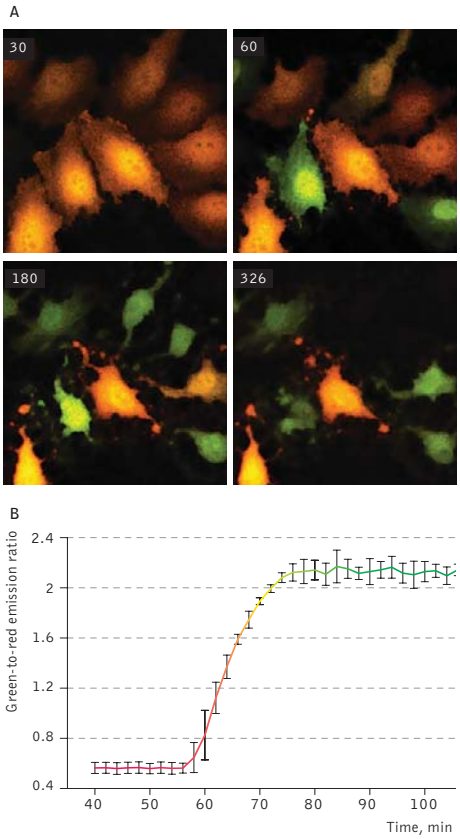
Change in excitation/emission spectra of TagBFP-TagGFP2 FRET pair upon the cleavage of the linker connecting two fluorescent proteins.

TagGFP2-TagRFP pair

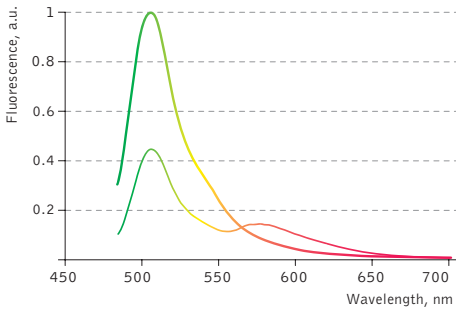
The high fluorescence quantum yield of TagGFP2 along with the high molar extinction coefficient of TagRFP and excellent overlap of donor emission and acceptor excitation spectra result in highly effective FRET between these fluorescent proteins. The calculated Forster distance R_0 = 5.7 nm for TagGFP2 - TagRFP pair is one of the largest among the values reported. At the same time, since TagGFP2 and TagRFP emission peaks are spaced by as much as 78 nm, the emission signal for these two proteins can be easily separated in any imaging system.

Shifting the wavelengths toward the red part of the spectrum (comparing to traditional cyan and yellow FRET partners) reduces input of cellular autofluorescence. The significantly increased quantum yield of TagRFP is highly beneficial for acceptor-based ratiometric FRET studies. High pH-stability of the both proteins allows using this pair for imaging in acidic organelles. The combined advantages suggest that the TagGFP2-TagRFP is one of the most efficient green/red FRET couple available to date for ratiometric FRET analyses. As an additional advance, TagRFP and TagGFP2 proteins derive from different marine sources and therefore luck the ability to form heterodimers. It ensures zero background for FRET analysis that may not be the case for weakly dimerizing FRET pairs consisting of highly homological fluorescent proteins. We believe that advantageous characteristics make TagGFP2-TagRFP one of the preferable FRET pairs to monitor interaction of proteins of interest in living cells and to generate FRET-based sensors of various specificity (see demonstration of FRET-based caspase-3 apoptosis sensor Casper3-GR performance and [Shcherbo *et al.*, 2009]).

The excitation wavelength required to visualize FRET changes of the TagGFP2-TagRFP pair by ratio-imaging is provided by an ordinary FITC/GFP excitation filter or ubiquitous 488 nm laser line, and the two emission signals are acquired using a 500-530 nm (FITC/GFP emission filter) bandpass filter and a 560-600 nm bandpass filter (Cy3/DsRed emission filter) or a 560LP longpass filter.



Green-to-red emission ratio change of Casper3-GR upon staurosporine-induced apoptosis. (A) Two channel fluorescence imaging of Casper3-GR. Time (in min.) is shown after staurosporine infusion. (B) Emission ratio shown for 5 cells, time point aligned to the median of ratio changes, individual for each cell. Excitation at 488 nm, emission was detected at 500-530 nm and 560-600 nm.



Emission spectra of Casper3-GR before (thin line) and after digestion by Caspase-3 (thick line).

References:

1. Shcherbo D, Souslova EA, Goedhart J, Chepurnykh TV, Gaintzeva A, Shemiakina II, Gadella TW, Lukyanov S, Chudakov DM. Practical and reliable FRET/FLIM pair of fluorescent proteins. BMC Biotechnol. 2009; 9:24. / pmid: 19321010

2. Subach OM, Gundorov IS, Yoshimura M, Subach FV, Zhang J, Gruenwald D, Souslova EA, Chudakov DM, Verkhusha VV. Conversion of Red Fluorescent Protein into a Bright Blue Probe. Chem Biol. 2008; 15 (10):1116-24. / pmid: 18940671

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