Procedures for Requesting BiFC Constructs

We have provided our mammalian BiFC and worm BiFC plasmids to several hundreds of research labs since 2003. To expedite the processing of a large number of requests, we have transferred our plasmids to Addgene for distribution. Please visit Addgene website and obtain the plasmids you need at the Chang-Deng Hu Lab Addgene Page. However, please contact me if you have any technical questions regarding our BiFC plasmids or BiFC methods. If you need to modify the MTA provided by Addgene, please contact our Sponsored Program Service at Purdue University.

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Visualization of Protein Interactions in Living Cells Using Bimolecular Fluorescence Complementation (BiFC) Assays

The following are suggested BiFC and multicolor BiFC protocols based on our experimental conditions. You may need to modify the experimental procedures based on cell lines and particular proteins you will work on. Detailed design of BiFC and multicolor BiFC constructs and experimental procedures can be found from our BiFC publications (Current Protocol in Cell Biology, Protein-Protein Interaction, and other BiFC publications).

BiFC Protocol

COS-1 cells are cultured in Dulbecco's Modified Eagle Medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum with penicillin and streptomycin in 12-well plates at 37 ^{o}C. The 0.25 ~0.50 mg of plasmids encoding two proteins fused to VN155(I152L) or VC155(A206K) are transfected into COS-1 cells using FuGene6 (Roche, Indianapolis, IN). Plasmids encoding one or two mutant fusion proteins should be transfected at the same time. For ratio analysis, 50 ng of plasmids encoding ECFP are cotransfected as an internal control to measure the BiFC efficiency of fragments derived from Venus. YFP and CFP images from the same fields of transfected cells are acquired 12-24 h after transfection using a CCD camera mounted on a Nikon inverted fluorescence microscope with JP4 filters (Chroma, Rockingham, VT). The intensity of more than 100 cells is individually quantified using an automated intensity recognition feature of Metamorph II (Universal Imaging Corporation, Downingtown, PA). YFP/CFP ratio of individual cells is calculated. To determine whether the BiFC signal represents specific interactions, the median of YFP/CFP ratios is determined in each group and used for comparison of the BiFC efficiency between the wild type and mutant fusion proteins. For details, please refer to Biotechniques (49:793-805, 2010).

Multicolor BiFC Protocol

The best combination for multicolor BiFC assay is Venus 1-172 (VN173), Cerulean 1-172 (CrN173) and ECFP 155-238 (CC155). Since 7.8% of the BiFC signal derived from VN173 paired with CC155 is read in the CFP channel, this signal crosstalk should be corrected by multiplying original CFP images with a correction coefficient. Briefly, cells transfected with plasmids encoding VN173 fused to one of your proteins and CC155 fused to another protein should be used as a control to determine the correction coefficient. Fluorescent images are captured with both YFP and CFP filters and the correction coefficient is calculated as described for FRET (Gordon, et al., 1998, Biophys J *74*:2702-2713).The original CFP images in the multicolor BiFC experiments should multiplied by the correction coefficient using FRET function provided in Metamorph II (Universal Imaging Corporation, Downingtown, PA) to obtain corrected CFP images for ratio analysis. Detailed multicolor BiFC analysis can be found from Current Protocol in Cell Biology (21.3.1-21.3.21) and other BiFC publications.

Critical Application Notes:

It is essential that you use a mutant protein in which a substitution or a small deletion is introduced into the interaction interface as a negative control in order to conclude that your BiFC signal represents specific interactions between the two proteins. Fragments alone (e.g. VN73, or VC155) of any fluorescent proteins can not be used as negative controls. In fact, coexpression of two fragments alone, or one fusion and one fragment, may result in higher background fluorescent signals due to nonspecific assembly. Detailed protocols and appropriate controls can be found from our BiFC publications.

Depending on the effect of mutations on the interactions of your proteins, you may see fluorescent cells in your control groups as well. Since BiFC complexes are essentially irreversible and the complexes can be accumulated over time, you may still see some fluorescent cells if you incubate for too long or proteins are highly overexpressed. For your convenience, we include representative images from our mutant control experiments that are presented in Figure 2 (BioTechnique, 40:61-66, 2006). Note that we deleted two heptads in the zipper region of bFos and there are still three heptads left in the fusion protein. The newly improved BiFC assay with the mutation I152L introduced into VN155 shows lower self-assembly when co-expressed with VC155. However, self-assembly is not eliminated. Thus, appropriate negative controls are needed in your BiFC experiments.

In addition, it is not clear whether the BiFC assays can be used to study dynamic interactions or not since the BiFC complexes are virtually irreversible in vitro (Molecular Cell, 9:789, 2002).

BiFC Publications

Pratt, E.P.S., Owens, J.L., Hockerman, G.H., and Hu, C.D. Bimolecular fluorescence complementation (BiFC) analysis of protein-protein interactions and assessment of subcellular localization in live cells. High resolution imaging of proeins in tissues and cells: light and electron microscopy methods and protocols (Ed, Schwartzbach, S.D., Skalli, O., and Schikorski, T.), Springer (2015).
(This is a BiFC protocol for confocal microscopy)

Kodama, Y. and Hu, C.D.* Bimolecular fluorescence complementation (BiFC) analysis of protein-protein interaction: How to calculate signal-to-noise ratio. *Methods Cell Biol*., 113: 107-121 (2013).
(This is the protocol using newly improved mutant fragments of Venus: VN155-I152L and VC155. This pair of BiFC cloning vectors should be used for most single pair of protein-protein interaction studies, and the protocol also provides detailed procedures for quantification of signal to noise ratio.)

Kodama, Y. and **Hu, C.D.**. An improved bimolecular fluorescence complementation assay with a high signal-to-noise ratio. *Biotechniques*, **49**:793-805 (2010)
(This improved BiFC assay has lower self-assembly of two non-fluorescent fragments of Venus and can be used to replace the previously developed Venus-based BiFC assay published in Biotechniques in 2006)

Shyu, Y. and **Hu, C.D.** Fluorescence complementation: an emerging tool for biological research. *Trends Biotechnol.* **26**:622-630 (2008)
(Review of BiFC-based technologies and applications)

Shyu, Y.,Fox, SM., Duren, HM., Ellis, R.E., Kerppola, T.K. and **Hu, C.-D.** Visualization of protein interaction in living Caenorhabditis elegans using bimolecular fluorescence complementation (BiFC) analysis. *Nat. Protoc.*, **4**:588-596 (2008).
(Protocols of worm BiFC assay for visualization of protein interactions in living worms)

Hiatt, S.M., Shyu, Y., Duren, H.M, and **Hu, C.D.** Bimolecular fluorescence complementation (BiFC) analysis of protein interactions in living *C. elegans*. *Methods*, **45**:185-191 (2008)
(Original report of worm BiFC assay for visualization of protein interactions in living worms)

Shyu, Y., Suarez, C., and **Hu, C.D.** Visualization of ternary complexes in living cells by using a BiFC-based FRET analysis. *Nat. Protoc*. **3**:1693-1702 (2008)
(Protocols of BiFC-FRET assay for visualization of protein interactions in living worms)

Shyu, Y., Suarez, C., and **Hu, C.-D**. Visualization of AP-1-NF-kB ternary complexes in living cells by using a BiFC-based FRET. *Proc. Natl. Acad. Sci. U.S.A*., **105**:151-156 (2008).

(Original report of BiFC-FRET assay for visualization of ternary complexes in living cells and the identification of p65-Jun-Fos ternary complex)

Liu, H., Deng, X., Shyu, Y., Li, J.J., Taparowsky, EJ., and **Hu, C.-D.** Mutual regulation of c-Jun and ATF2 by transcriptional activation and subcellular localization. *The EMBO Journal,* **25**:1058-1069 (2006).
(Applications of the improved BiFC system in visualization of AP-1 protein interactions)

Shyu, Y., Liu, H., Deng, X., and **Hu, C.-D**. Identification of new fluorescent fragments for BiFC analysis under physiological conditions. *BioTechniques*, **40**:61-66 (2006).
(Original report and detailed experimental procedures of the improved BiFC assay)

Hu, C-D., Grinberg A., and Kerppola T. Visualization of protein interaction in living cells using bimolecular fluorescence complementation (BiFC) analysis. *Current Protocol in Cell Biology*. 21.3.1-21.3.21, 2005.
(Applications and detailed experimental procedures of the multioclor BiFC assay in visualization of Myc/Max family of protein interactions)

Hu, C-D. and Kerppola TK. Direct visualization of protein interactions in living cells using bimolecular fluorescence complementation analysis. *Protein-Protein Interactions* (ed. P. Adams and E. Golemis), Cold Spring Harbor Laboratory Press, 2005.
(Detailed design and experimental procedures of BiFC and multicolor assays in general)

Grinberg A, **Hu, C.-D**., and Kerppola T. Visualization of Myc/Max/Mad family dimers and the competition for dimerization in living cells. *Mol. Cell. Biol*. **24**, 4294-4308 (2004).
(Detailed design and experimental procedures of BiFC and multicolor BiFC assays in general)

Hu, C.-D. and Kerppola, T. Simultaneous visualization of interactions between multiple proteins in living cells using multicolor bimolecular fluorescence complementation analysis. *Nat. Biotechnol.* **21**, 539-545 (2003).
(Original report of multicolor BiFC assay and its applications)

Hu, C.-D. Chinenov, Y., and Kerppola, T Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. *Mol. Cell*. **9**, 789-798 (2002).
(Original report of BiFC assay and its applications)

BiFC Vectors, Sequences, and Maps

Download Sequences and Maps

Sequences	Maps
pBiFC-VN173	pBiFC-VN173
pBiFC-VC155	pBiFC-VC155
pBiFC-bJunVN173	pBiFC-bJunVN173
pBiFC-bFosVC155	pBiFC-bFosVC155
pBiFC-bFos(deltaZIP)VC155	pBiFC-bFos(deltaZIP)VC155
pBiFC-CrN173	pBiFC-CrN173
pBiFC-CC155	pBiFC-CC155
pCE-BiFC-VN173	pCE-BiFC-VN173
pCE-BiFC-VC155	pCE-BiFC-VC155
pBiFC-VN155(I152L)	pBiFC-VN155(I152L)
pBiFC-bJunVN155(I152L)	pBiFC-bJunVN155(I152L)

BiFC Vectors

Cloning Vectors for BiFC Assay

1. pBiFC-VN173 (pFLAG-CMV backbone)
2. pBiFC-VC155 (pCMV-HA backbone)

Cloning Vectors for Multicolor BiFC Assay

1. pBiFC-VN173 (pFLAG-CMV backbone)
2. pBiFC-CrN173 (pFLAG-CMV backbone)
3. pBiFC-CC155 (pHA-CMV backbone)

Positive and Negative Controls for BiFC Assay

1. pBiFC-bJunVN173 (pFLAG-CMV backbone)
2. pBiFC-bFosVC155 (pCMV-HA backbone)
3. pBiFC-bFos(deltaZIP)VC155 (pCMV-HA backbone)

Improved BiFC plasmids

1. pBiFC-VN155(I152L) (pCMV-Myc backbone, replacement of pBiFC-VN173)
2. pBiFC-bJunVN55(I152L) (pCMV-Myc backbone, replacement of pBiFC-bJunVN173)

Cloning Vectors for Worm BiFC Assay

1. pCE-BiFC-VN173
2. pCE-BiFC-VC155