Bimolecular Fluorescence Complementation (BiFC) Assay for Determination of in-vitro Protein-Ligand Interaction using the Sapphire Biomolecular Imager

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Introduction

Bimolecular Fluorescence Complementation (BiFC) assay is a method used to directly visualize protein-protein interaction in cells. Venus fluorescent protein (VFP) has been identified as the most useful yellow fluorescent protein for BiFC analysis (1). When two separated fragments of the full-length protein, representing the N-terminus (VN173, amino acids 1–173) and the C-terminus (VC155, amino acids 152–239), are close enough, they will regenerate the active form VFP that is used as a reporter.

This app note explores the application of the BiFC assay to the study of in vitro interaction between the Zika virus NS4B protein and its ligand, TBK1. To determine whether these proteins interact, each was combined with a unique fragment of VFP. NS4B was coupled to the C-terminal fragment of VFP, while TBK1 was coupled to the N-terminal fragment. If NS4B and TBK1 interact as expected, the two would come in close enough contact to allow regeneration of VFP, producing yellow fluorescent signal as detected by the Sapphire Biomolecular Imager.

Positive and negative controls were included in the study. NS4B is known to form homodimers (2). To create a positive control, NS4B was combined with both the N-terminal and C-terminal fragments of VFP. Upon homodimerization, VFP would regenerate and produce fluorescent signal. As a negative control, cells were transfected with NS4B fused with the N-terminal fragment of VFP alone. As only one fragment of VFP was present in the sample, no fluorescent signal would be generated.

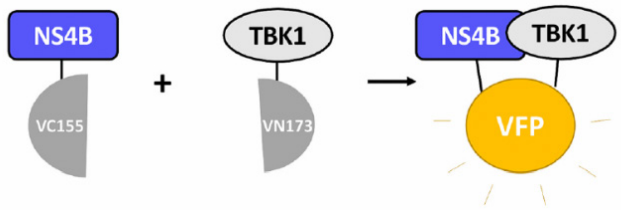

Figure 1. Schematic representation of BifC constructs. N-terminus (VN173) or the C-terminus (VC155) of the Venus fluorescent protein (VFP) were fused to TBK1 and NS4B, respectively. If the proteins are in close proximity, the active form VFP will be reconstituted, serving as reporter.

Materials and Methods

Full-length NS4B was PCR-amplified with specific primers. For chimeric NS4B-VN173 and NS4B-VC155, the complete sequence of NS4B was cloned in pBiFC-VN173, and at in pBiFC-VC155. The TBK1 sequence was fused with VN173 at its C-terminus (3). Previously reported homodimerization of NS4B (2) was used as a positive interaction model for BiFC complementation. The degree of protein interaction was measured by scanning with the Sapphire Biomolecular Imager

Plasmids pBiFC-VC155 (Addgene plasmid # 22011; RRID: Addgene_22,011) and pBiFC-VN173 (Addgene plasmid # 22010; RRID:Addgene_22,010) were gifts from Chang-Deng Hu (1).

1. In a flat bottom 24-well plate, HeLa cells were seeded at a density of 1.2x105 cells/ml in complete medium (DMEM + 10% fetal calf serum). Cells were left to adhere to the surface for 16-24 h at 37 °C, 5% CO_2.

2. For co-transfection with the fusion plasmids, DNA: Lipofectamine 3000 (Invitrogen) complexes were prepared according to manufacturer's instruction.

3. Culture media was removed and replaced with incomplete medium (DMEM).

4. DNA complexes were added to each well and incubated at 37 °C, 5% CO_2 to allow cellular entry. A final DNA concentration of 0.5 µg/well was used.

5. Three hours post-transfection, 10% FBS was supplemented to each well. Cells were incubated for 24 h at 37 °C in a cell culture incubator.

6. 48 h post-transfection, cells were rinsed with phosphate buffered saline (PBS) and left in PBS for fluorescence detection. Alternatively, cells can be fixed with 2% paraformaldehyde for longer storage.

7. The plates were scanned on the Sapphire using the 520 nm channel. The focus type was set to Plate (+3.00 mm), intensity was set to 10, and resolution was set to 20 µm.

Results

Figure 2

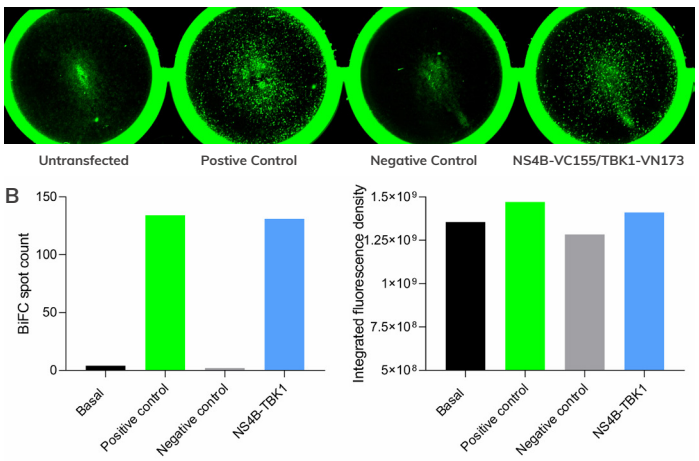

Figure 2. BifC assay results (A) Fluorescence image of BifC assay in 24-well plate obtained with Azure Sapphire Biomolecular Imager, using excitation and emission wavelengths of 520/565 nm. (B) Graph representing BiFC spot count (left) and lintegrated fluorescence density (right) calculated for each treatment: Basal (untransfected), Positive control (NS4B-VC155 and NS4B-VN173), Negative control (NS4B-VN173). Experimental sample (NS4B-VC155/TBK1-VN173). Analysis was performed with ImageJ software.

Conclusion

The Sapphire makes it possible to detect bimolecular fluorescence complementation spots from transfected adherent cells grown in clear plastic well plates. To demonstrate this application, the interaction between NS4B and TBK1 was monitored. Cells were transfected with the fused constructs and the fluorescence signal of the active form of VFP, serving as a reporter, was detected. This methodology may be suitable for in -vitro screening of protein interactions in living cells.

Acknowledgements

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References

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