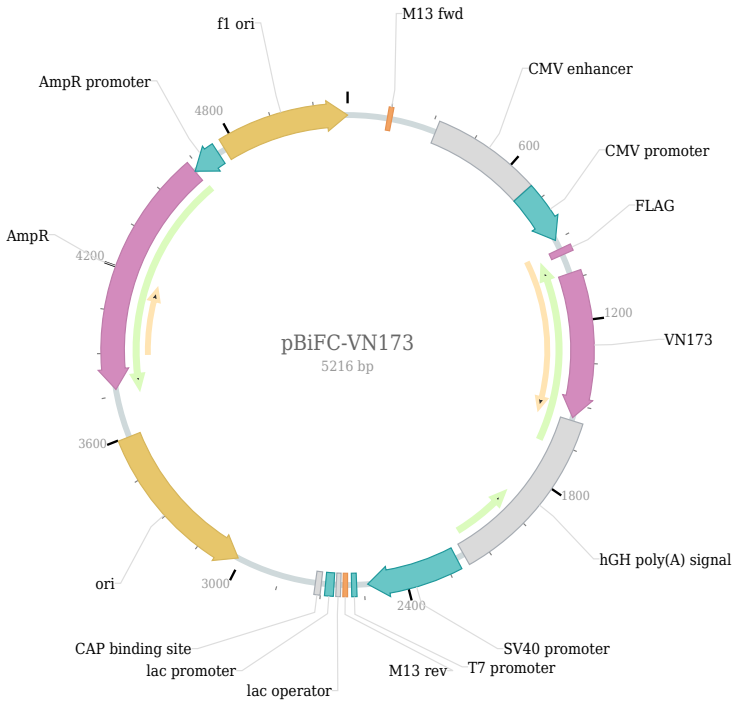
pBiFC-VN173

Vector ID	V012099
Antibiotic Resistance	Ampicillin
Length	5216 bp
Vector Type	Bimolecular fluorescent complementation vectors (B

Product Information

Replication Origin	ori
Selection Marker	VN173
Copy Number	High copy number
Promoter	CMV
Cloning Method	Restriction Enzyme
Fusion Tag	Flag
Growth Strain(s)	DH10B
Growth Temperature	37℃
Comment	pBiFC-VN173 is designed for mammalian expression of a Venus-derived fluorescent protein fragment, enabling BiFC and multicolor BiFC assays to study protein-protein interactions with high sensitivity and versatility in living cells. Objective: To construct pBIFC-VN173-CXCR4 and pBIFC-VC155-NT21MP eukaryotic expression plasmids and to investigate the interaction of chemokine receptor 4 (CXCR4) and viral macrophage inflammatory protein-II(vMIP-II) N-terminal 21 peptides (NT21MP) in living cells. Methods: DNA fragment encoding NT21MP was chemically synthesized and inserted into the BiFC eukaryotic expression vector pBIFC-VC155. The full length of CXCR4 DNA fragment was amplified by RT-PCR from SKBR (3) cells and inserted into BiFC eukaryotic expression plasmid pBIFC-VN173. Two recombinant vectors were identified by restriction enzyme digestion and DNA sequencing. The recombinant vectors were cotransfected into Africa green monkey kidney fibroblast COS-7 cells by using Lipofectamine 2000. The interaction of NT21MP and CXCR4 was detected by bimolecular fluorescence complementation (BiFC) assay. Results: The restriction enzyme digestion and DNA sequences and open read frames of two vectors were consistent with experiment design. The BiFC plasmids were successfully cotransfected into the target cells and expressed. The strong BiFC signals were detected in pBIFC-VN173-CXCR4 and pBIFC-VC155-NT21MP cotransfected cells and the fluorescence signal was located in the cytoplasm. Conclusion: The eukaryotic expression plasmids for BiFC assay are successfully constructed. The interaction of NT21MP and CXCR4 in living cells can be detected by using this technology.

Vector Map



Plasmid Resuspension Protocol

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 μl for 5 μg plasmid, and 100 μl for 100 μg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.