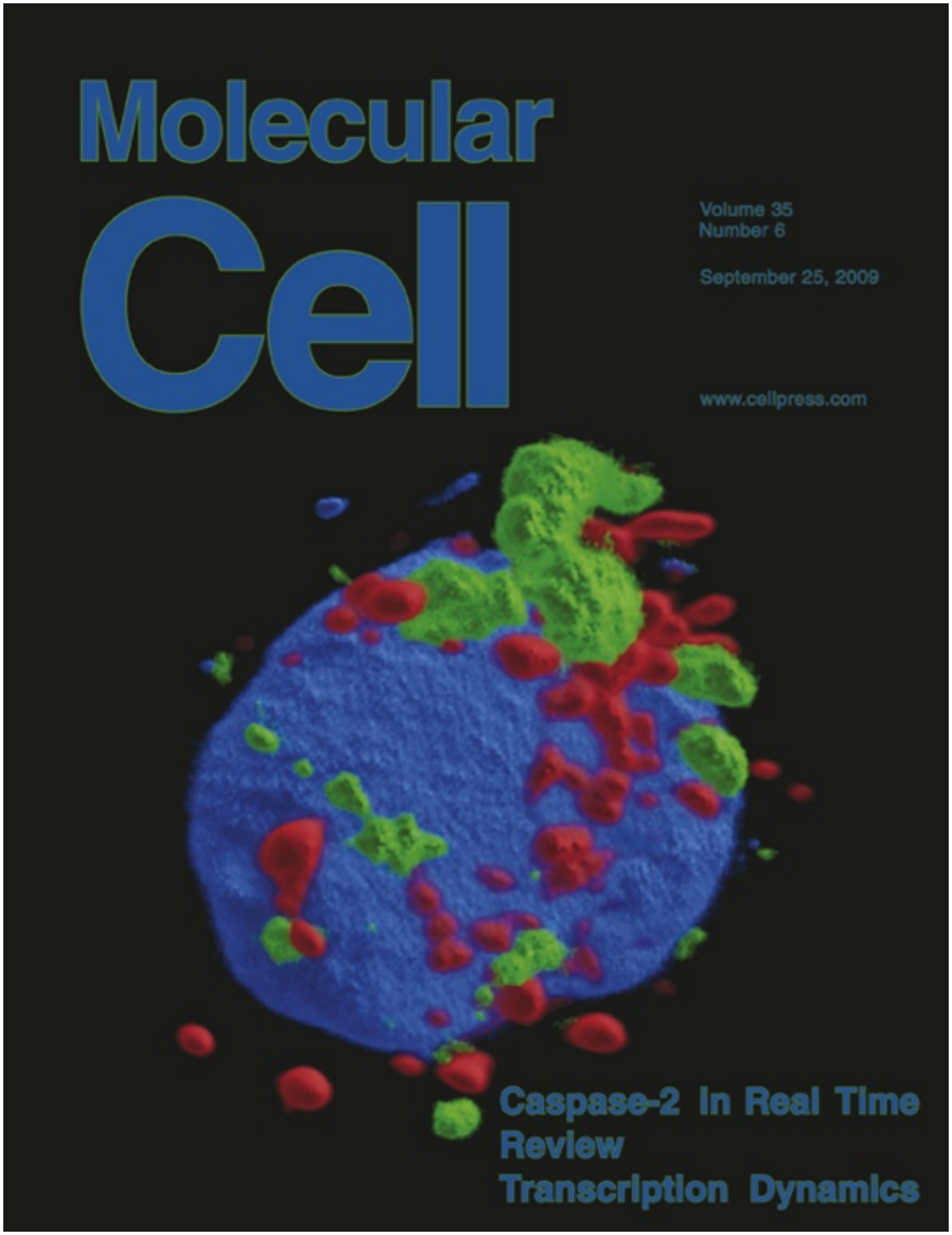
Caspase-2 BiFC plasmids



Caspase-2 BiFC - background

Bimolecular Fluorescence Complementation (BiFC) describes the use of split fluorescent proteins to measure protein-protein interactions in cells. The split fluorescent protein fragments are not fluorescent by themselves, but when they are fused to interacting proteins they can associate to form the fluorescent molecule (Shyu et al. Biotechniques, 2006).

The plasmids use split Venus (a brighter, more photostable variant of YFP) to measure caspase-2 recruitment to its activation platform.

We fused caspase-2 to the N-terminal half (VN) and to the C-terminal half of Venus (VC). Following recruitment of caspase-2 to its activation platform, induced proximity of caspase-2 occurs and, consequently, association of the two halves of Venus is enforced. Induced proximity of caspase-2 is required for its dimerization and can be measured as increased Venus fluorescence by confocal or fluorescent microscopy.

Please reference these plasmids by citing the following article in any publications.

Bouchier-Hayes L, Oberst A, McStay GP, Connell S, Tait SW, Dillon CP, Flanagan JM, Beere HM, Green DR. Characterization of cytoplasmic caspase-2 activation by induced proximity. Mol Cell. (2009) 35:830-40.

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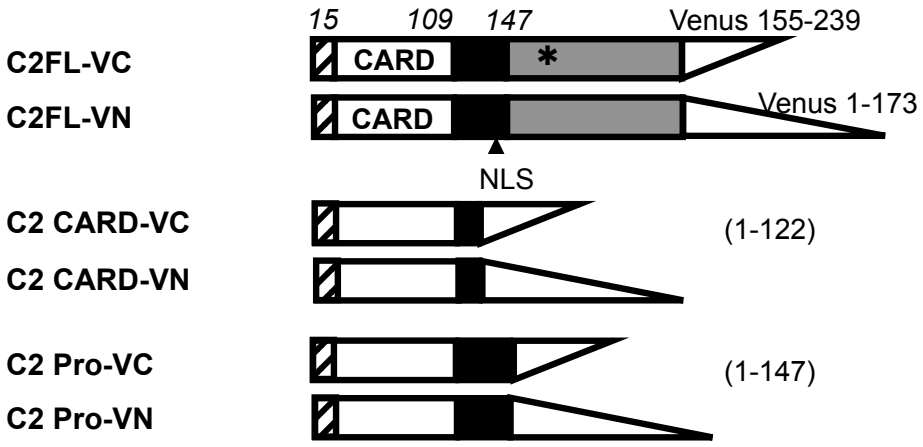
Materials Available



1. **C2FL-VC**: pBiFC-HA-Caspase 2 FL (C303A)-VC155. Catalytically inactive (*PF2424*) Addgene 49257
2. **C2FL-VN**: pBiFC-FLAG-Caspase 2 FL (C303A)-VN173. Catalytically inactive (*PF2425*) Addgene 49258
3. **C2 CARD-VC**: pBiFC-HA-Caspase 2 CARD-VC155. amino acids 1-122 (*PF2398*) Addgene 49259
4. **C2 CARD-VN**: pBiFC-FLAG-Caspase 2 CARD-VN173. amino acids 1-122 (*PF2397*) Addgene 49260
5. **C2Pro-VC**: pBiFC-HA-Caspase 2 prodomain-VC155. amino acids 1-147 (*PF3000*) Addgene 49261
6. **C2Pro-VN**: pBiFC-FLAG-Caspase 2 prodomain-VN173. amino acids 1-147 (*PF3001*) Addgene 49262

***Figure**: Schematic representation of the caspase-2 BiFC constructs. (*) indicates the active cysteine that was mutated to alanine in C2FL. NLS= Nuclear localization signal*

Caspase-2 Sequence

ACCESSION U13021

ATGGCCGCTGACAGGGGACGCAGGATATTGGGAGTGTGTGGCATGCATCCTCATCATCAGGAAAC
TCTAAAAAAGAACCGAGTGGTGCTAGCCAAACAGCTGTTGTTGAGCGAATTGTTAGAACATCTTCT
GGAGAAGGACATCATCACCTTGGAAATGAGGGAGCTCATCCAGGCCAAAGTGGGCAGTTTCAGC
CAGAATGTGGAACTCCTCAACTTGCTGCCTAAGAGGGGTCCCCAAGCTTTTGATGCCTTCTGTGA
AGCACTGAGGGAGACCAAGCAAGGCCACCTGGAGGATATGTTGCTCACCACCCTTTCTGGGCTT
CAGCATGTACTCCCACCGTTGAGCTGTGACTACGACTTGAGTCTCCCTTTTCCGGTGTGTGAGTC
CTGTCCCCTTTACAAGAAGCTCCGCCTGTCGACAGATACTGTGGAACACTCCCTAGACAATAAAGA
TGGTCCTGTCTGCCTTCAGGTGAAGCCTTGCACTCCTGAATTTTATCAAACACACTTCCAGCTGGC
ATATAGGTTGCAGTCTCGGCCTCGTGGCCTAGCACTGGTGTTGAGCAATGTGCACTTCACTGGAG
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CTTTTGGGCTATGACGTCCATGTTCTATGTGACCAGACTGCACAGGAAATGCAAGAGAAACTGCAG
AATTTTGCACAGTTACCTGCACACCGAGTCACGGACTCCTGCATCGTGGCACTCCTCTCGCATGG
TGTGGAGGGCGCCATCTATGGTGTGGATGGGAAACTGCTCCAGCTCCAAGAGGTTTTTCAGCTCT
TTGACAACGCCAACTGCCCAAGCCTACAGAACAAACCAAAAATGTTCTTCATCCAGGCCTGCCGT
GGAGATGAGACTGATCGTGGGGTTGACCAACAAGATGGAAAGAACCACGCAGGATCCCCTGGGT
GCGAGGAGAGTGATGCCGGTAAAGAAAAGTTGCCGAAGATGAGACTGCCCACGCGCTCAGACAT
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ACATCGAGGCTCTTGCTCAAGTGTTTTCTGAGCGGGCTTGTGATATGCACGTGGCCGACATGCTG
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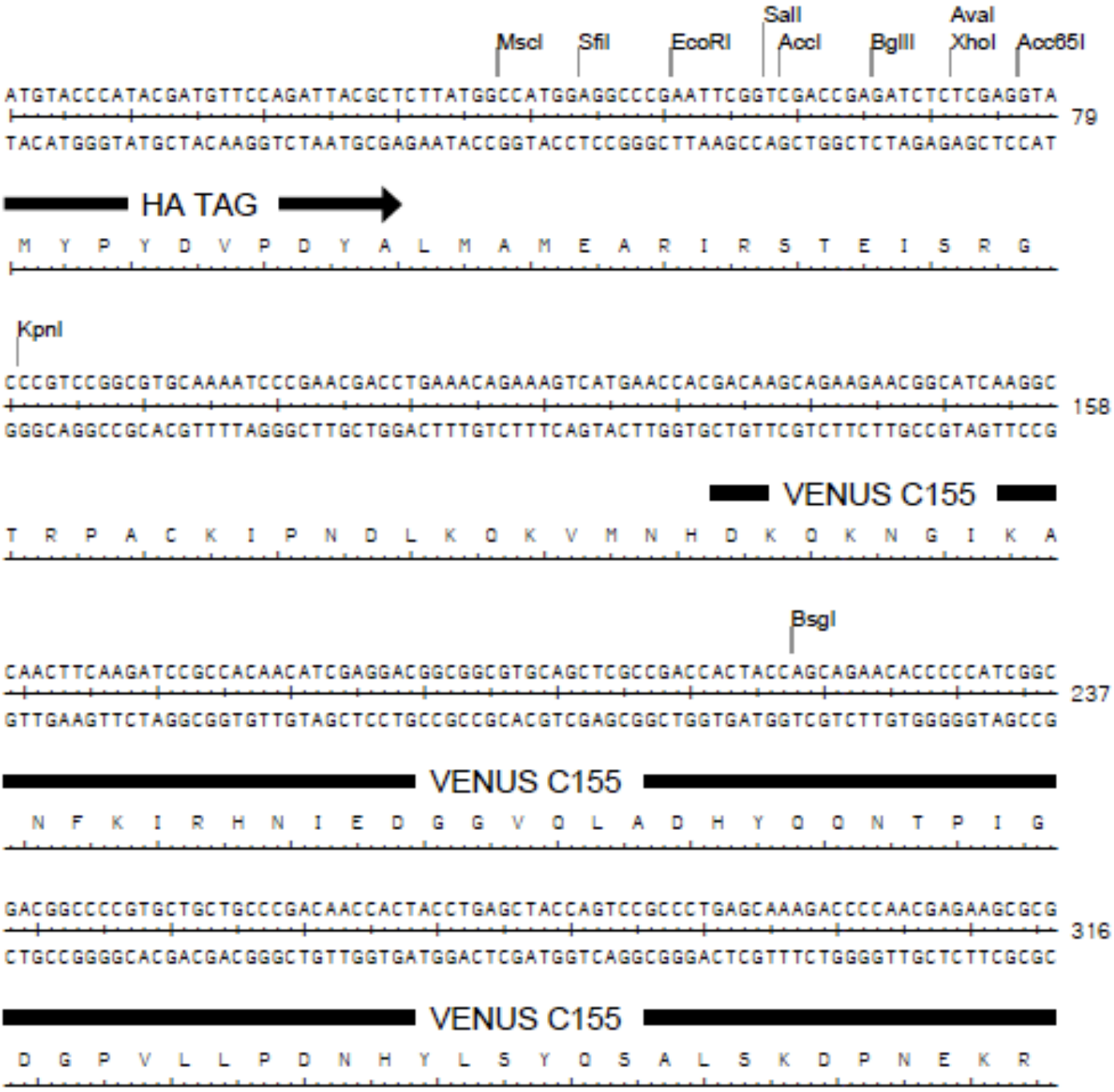
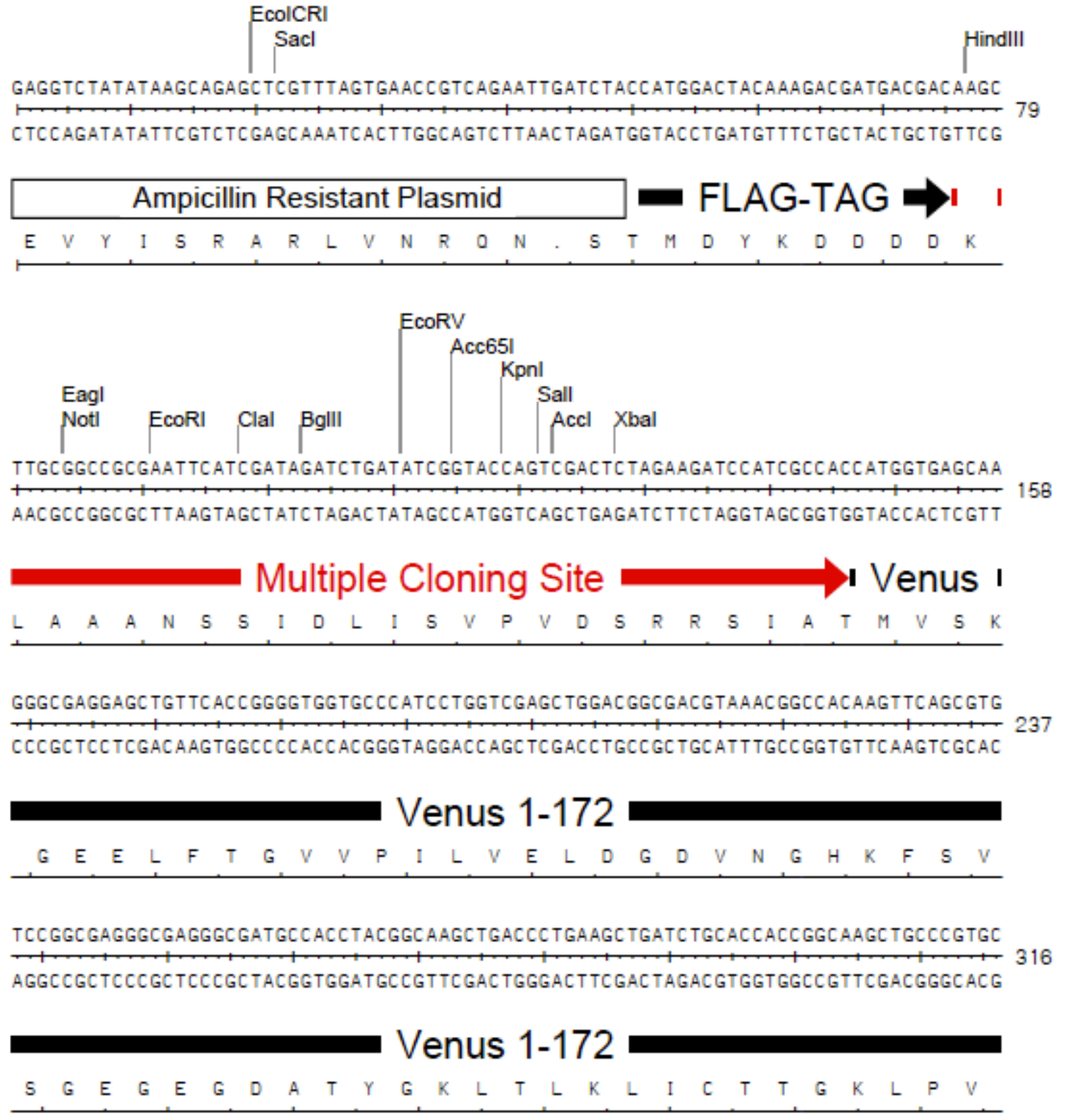
CCACATGA

pBiFC VC155 MCS



For more info on the BiFC constructs see Chang-Deng Hu website: http://people.pharmacy.purdue.edu/~cdhu

pBiFC VC173 MCS



For more info on the BiFC constructs see Chang-Deng Hu website: http://people.pharmacy.purdue.edu/~cdhu

Cloning

The Following restrictions sites were used to clone the Caspase-2 fragments into the BiFC vectors:

1. **C2FL-VC**: pBiFC-HA-Caspase 2 FL (C303A)-VC155. Catalytically inactive (*PF2424*)

   BglII/BglII

2. **C2FL-VN**: pBiFC-FLAG-Caspase 2 FL (C303A)-VN173. Catalytically inactive (*PF2425*).

   BglII/XbaI

3. **C2 CARD-VC**: pBiFC-HA-Caspase 2 CARD-VC155. amino acids 1-122 (*PF2398*)

   BglII/BglII

4. **C2 CARD-VN**: pBiFC-FLAG-Caspase 2 CARD-VN173. amino acids 1-122 (*PF2397*)

   BglII/BglII

5. **C2Pro-VC**: pBiFC-HA-Caspase 2 prodomain-VC155. amino acids 1-147 (*PF3000*)

   Eco RI/ SalI

   Note: C2 Pro VC nucleotide sequence contains silent mutations – the amino acid sequence is the same (made as an intermediate to make a different plasmid) but the nucleotide sequence is different:

   ATGGCTGCCGATCGTGGCCGGAGAATTTTAGGCGTATGTGGAATGCACC
   CCCACCACCAAGAGACCCTGAAGAAAAACAGGGTCGTGCTGGCTAAGCA
   ATTGCTGCTGTCCGAGCTCCTGGAGCACTTGCTTGAAAAAGATATTATTA
   CACTGGAGATGAGAGAACTGATTCAAGCTAAGGTTGGGAGCTTTAGCCA
   AAACGTCGAGTTGTTGAATCTGTTGCCCAAAAGAGGACCTCAGGCCTTCG
   ACGCTTTCTGCGAGGCTTTGAGAGAAACTAAACAGGGGCATTTGGAAGA
   CATGCTGCTGACTACTTTGTCAGGACTGCAACACGTGCTGCCTCCTCTGT
   CATGCGATTATGATCTGAGTTCTCTTCCCTTCCCTGTTTGCGAATCCTGCC
   CTCTCTATAAAAAACTGAGGCTCTCTACCGACACCGTGGAGCATTC

6. **C2Pro-VN**: pBiFC-FLAG-Caspase 2 prodomain-VN173. amino acids 1-147 (*PF3001*)

   Eco RI/ XbaI

Transfection conditions

The fusion proteins are expressed by transient transfection. Typically 20ng - 40ng of each of the C2-CARD pair or C2-Pro pair is transfected into Hela cells per well of a 6 well plate. The relative expression of C2-FL is somewhat lower, therefore more plasmid should be transfected (100-200ng). Too high expression will increase the non-specific refolding of the Venus fragments, therefore it is important to assess background levels of BiFC under conditions of no treatment in your cell line of choice and titrate the plasmid amounts accordingly.

MEFs require higher amounts of plasmid (most likely due to lower overall transfection efficiency). 250-500ng in a well of a 6 well plate works well.

It is recommended to use a fluorescent reporter, such as pshooter.dsRed-mito, to label the transfected cells.

We use lipofectamine 2000 transfection reagent for transfection of Hela and MEF cells.

Microscopy

The BiFC fusion proteins can be viewed by confocal or fluorescent microscopy. We have successfully imaged BiFC using the following microscopes

1) Marianas Spinning Disc Confocal imaging system (Intelligent Imaging Innovations/3i, Denver, CO) and a Carl Zeiss 200M motorized inverted microscope (Carl Zeiss MicroImaging, Thornwood, New York), equipped with spherical aberration correction optics (3i). Venus is excited using a 523nm laser (YFP). Venus can also be excited using a GFP laser (eg 473nm). Note, due to overlapping spectra you cannot visualize GFP and YFP simultaneously.

2) Marianas fluorescent microscope using the GFP filter of a mercury lamp