

pLV_ Hef1a/LacO -MashI-EGFP-Ubc-Hyg

Backbone: pLV-Hef1a-LacOid-NheI-Ngn3-T7-IRES2-DsRed2

Insert: SfiI_MashI-EGFP-Ubc-Hyg_BstEII

Parts:

MashI (from pLV-TRE-MashI-IRES2-EGFP):

atggagagctctggcaagatggagagtggagccggccagcagccgcagccccgcagcccttctgcctcccgcagc
ctgcttctttgcgaccgcggcgggcgggcagcggcgggcgggccgcagctcagagcgcgcagcagcaacagccgc
agggcgccgcgcagcagggcgccgcagctgagccccggggcgacagccagccctcagggggcggtcacaagtgcagc
gccaagcaggtcaagcgcgcagcgcctcctcctccggaactgatgcgctgcaaacgcgggtcaacttcagcggctt
cggctacagcctgccacagcagcagccggccgcgctggcgcgccgcaacgagcgcgagcgcgaaccgggtcaagttgg
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ggcggggcgctcctgctgccccaccatctcccccaactactccaacgacttgaactctatggcgggttctccgggtcct
cctactcctccgacgagggatcctacgaccctcttagcccagaggaacaagagctgctggactttaccaactgggtc
tga

EGFP (from pLV-TRE-IRES2-EGFP):

atggtgagcaagggcgaggagctgttaccgggggtggtgcccatcctggctcgagctggacggcgacgtaaacggcca
caagttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgacctgaagttcatctgcaccaccg
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gccgacaagcagaagaacggcatcaaggtgaacttcaagatccgccacaacatcgaggacggcagcgtgcagctcgc
cgaccactaccagcagaacacccccatcggcgacggccccgctgctgctgcccgacaaccactacctgagcaccagct
ccgccctgagcaaaagacccccaaacgagagcgcgatcacatggtcctgctggagttcgtgacccgcgcgggatca
ctcggcatggacgagctgtacaagttaa

Ubc (from pLV-Ubc-IRES2-Hyg):

gatctggcctccgcgcgggttttggcgctcccgcgggcgccccctcctcacggcgagcgcctgccacgtcagacg
aagggcgagggagcgtcctgatccttccgcccggacgctcaggacagcggccccgctgctcataagactcggccttag
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cagtgttagactagtaaattgtccgctaaattctggccggttttggctttttgtagacgaag

Hyg (from pLV-Ubc-IRES2-Hyg):

atgaaaaagcctgaactcaccgcgacgtctgtcgcgagaagtttctgatcgaaaagttcgacagcgtctccgacctgat
gcagctctcggagggcgaagaatctcgtgctttcagcttcgatgtaggagggcgtggatatgtcctgcgggtaaata
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2A:

ggatctggcgccaccaacttctctctgctgaagcaggccggcgacgtggaggagaaccaggccca

helical linker:

ggcaacaacggcggcaacaacaacggcggctccaccggt

- MashI forward primer (MashI_fwd_EcoRI_2008-07-08)

gcatgggcccattacggccgccaccatggagagctctggcaagat

- MashI reverse primer (MashI_rev_helixNostop_2008-07-17)

(fw) gctggactttaccaactggttcggcaacaacggcggcaacaacaacgg

Reverse Compliment: ccgttggtgtgcccgcggtgtgtgcccgaaccagttggttaaagtccagc

- EGFP forward primer (EGFP_forward_helix_2008-07-17)

Ggcaacaacaacggcggctccaccggtatggtgagcaagggcgaggagctg

- EGFP reverse primer (EGFP_rev_stop_XcmI_2008-07-17)

(fw) tcggcatggacgagctgtacaagtaaccagggtggggattggatcgggaagatctgg

Reverse Compliment: ttcccgatccaatcccacctggttattacttgtacagctcgtccatgcc

gaagacgcttgtgagggcgggctgtgaggtcgttgaacaaggtggggggcatggtgggcggaagaacccaaggtct
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