

## pLV\_TRE\_SfiI\_Ngn1-EYFP-2A-mKate\_SfiI\_Ubc\_Bla

**Backbone:** pLV\_TRE\_SfiI\_Sox17\_SfiI\_Ubc\_Bla

**Insert:** SfiI\_Ngn1-EYFP\_SfiI

## Parts:

### Ngn1:

atgcctgcccctttggagacctgcatctctgatctcgactgctccagcagcaacagcagcagcgacctgtccagctt  
cctcaccgacgaggaggactgtgccaggctacagcccctagcctccacctcggggctgtccgtgccagcccggagga  
gcgctcccgcctctccggggcatcgaatgttcccggtgccaggacgaagagcaggaacggcggaggcggcgaggt  
cgcgctcgggtgcggctccgaggetctgctgcaactccctgcgaggagtcgctcgcgtcaaagccaacgatcgcgagcg  
caaccgcatgcacaacctcaacgctgcgctggacgccttgcgagcgtgctgccctcgttccccgacgacaccaagc  
tcaccaagattgagacgctgcgcttcgcctacaactacatctgggcccctggctgagacactgcgctggcagatcaa  
gggctccccgggggagtgcccgggagcgcctcctgcctccgcagtggtccccctgtctgccgggccccgagccc  
ggccagcgacactgagtcctgggggtccggggcgcgctgctccccctgcccactgtggcatcaccactctctgacc  
ccagtagtccctcggcttcagaagacttcacctatggcccgggagatccccctttctcctttctctggcctgccc  
aacctgctccacacgacgcgcctgtttcatcccataaccactag

- Ngn1 forward primer (prIG08\_02\_SfiI\_Kz\_Ngn1)

gcatggggccattacggcctgccaccatgcctgcccctttggagac

- Ngn1 reverse primer (prIG08\_03r\_Ngn1\_link)

accggtggagcccgctgtgttgccgcgctgtgtgccgtggatgggatgaaacaggg

(fw) ccctgtttcatcccataaccaggaacaacggcggcaacaacaacggcggctccaccggt

### EYFP:

atggtgagcaagggcgaggagctgttcaccgggggtggtgcccatcctggctcgagctggacggcgacgtaaacggcca  
caagttcagcgtgtccggcgagggcgagggcgatgccacctacggcaagctgacctgaagttcatctgcaccaccg  
gcaagctgcccgtgccctggcccaccctcgtgaccaccttcggctacggcctgcagtgcttcgcccgtaccccgac  
cacatgaagcagcagcacttcttcaagtcgcgcatgcccgaaggetacgtccaggagcgcaccatcttcttcaagga  
cgacggcaactacaagaccgcgccgaggtgaagttcgagggcgacaccctgggtgaaccgcatcgagctgaagggca  
tcgacttcaaggaggacggcaacatcctggggcacaagctggagtacaactacaacagccacaacgtctatatcatg  
gccgacaagcagaagaacggcatcaaggtgaacttcaagatccgccacaacatcgaggacggcagcgtgcagctcgc  
cgaccactaccagcagaacacccccatcggcgacggccccgtgctgctgccgacaaccactacctgagctaccagt  
ccgcctgagcaaacgagaagcgcgatcacatggtcctgctggagttcgtgaccgcccgggagatcact  
ctcggcattggacgagctgtacaagtaa

Teal = stop codon

- **EYFP forward primer (prIG08\_04\_link-EYFP)**

ggcaacaacaacggcggctccaccggtatggtgagcaagggcgagg

- **EYFP reverse primer (prIG08\_05\_EYFP-2A)**

(fw) cggcatggacgagctgtacaagGggtcgggagctacaaatTTTTCC

Reverse complimentary:

ggaaaaatTTGTAGCTCCGGACCCCTGTACAGCTCGTCCATGCCG

**2A:**

Ggatctggcgccaccaacttctctctgctgaagcaggccggcgacgtggaggagaaccaggccca

**mKate:**

atggtgtctaagggcgaagagctgattaaggagaacatgcacatgaagctgtacatggagggcaccgtgaacaacca  
ccacttcaagtgcacatccgagggcgaaggcaagccctacgagggcaccagaccatgagaatcaaggtggtcgagg  
gcgccctctcccccttcgcttcgacatcctggctaccagcttcatgtacggcagcaaaaccttcatcaaccacacc  
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cacatacagatccaagaaaccgctaagaacctcaagatgcccggcgtctactatgtggacagaagactggaaagaa  
tcaaggaggccgacaaagagacctacgtcgagcagcagaggtggctgtggccagatactgcgacctccctagcaaa  
ctggggcacaacttaat

- **mKate forward primer (prIG08\_06\_2A-mKate)**

GggtccggagctacaaatTTTTCCctcctcaaacaggctggagatgtcgaagaaaatcctgggcctatggtgtctaagggcgaagagctgattaag

- **mKate reverse primer (prIG08\_07\_mKate\_SfiI)**

ccctagcaaaactggggcacaacttaattaaggccgctcggccatcgataag

**Final Product:**

gcatggggccattacggcctgccaccatgcctgcccctttggagactgcatctctgatctcgactgctccagcagc  
aacagcagcagcgcacctgtccagcttctcaccgacgaggaggactgtgccaggctacagcccctagcctccacctc  
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