

# pLV-TRE-GLRA1-Ubc-Neo

Backbone: pLV-TRE\_EcoRI\_IRES2-EGFP\_BsrGI

Insert: EcoRI\_GLRA1-Ubc-Neo\_BsiWI

## Parts:

### GLRA1 (from NCBI):

```
atgtacagcttcaatactcttgcactctacctttgggagaccattgtattcttccagccttgctgcttctaaggaggc
tgaagctgctcgtccgcacccaagcctatgtcaccctcggatttccctggataagctaatggggagaacctccggat
atgatgccaggatcaggcccaattttaaagggtccccagtgaaactgagctgcaacattttcatcaacagctttggt
tccattgctgagacaacctaggactataggggtcaacatcttccctgctggcagcaatggaacgacccccgcctggccta
taatgaataccctgacgactctctggacctggacctatccatgctggactccatctggaaacctgacctgttctttg
ccaacgagaagggggcccacttccatgagatcaccacagacaacaaattgctaaggatctcccggaaatgggaaatgctc
ctctacagcatcagaatcaccctgacactggcctgccccatggacttgaagaatttccccatggatgtccagacatg
tatcatgcaactggaaagctttggatatacgaatgacactcatctttgagtgaggaggaacagggagccgtgcagg
tagcagatggactaactctgccccagtttatacttgaaggagaaggacttgagatactgcaccaagcactacaac
acaggtaaattcacctgcattgaggcccggttccacctggagcggcagatgggttactacctgattcagatgtatata
tcccagcctgctcattgtcactctctcatggatctctctctggatcaacatggatgctgcacctgctcgtgtgggccc
taggcatacaccactgtgctcaccatgaccacccagagctccggctctcagagcatctctgcccagggtgctcctatgtg
aaagccattgacatttggatggcagtttgcctgctctttgtgttctcagccctattagaataatgctgcccgttaactt
tgtgtctcggcaacataaggagctgctccgattcaggaggaagcggagacatcacaaggaggaatgaagctggagaag
gcccgttttaacttctctgctatgggatgggcccagcctgtctacaggccaaggatggcatctcagtcaggggcgcc
aacaacagtaacaccaccaacccccctcctgcaccatctaaagtccccagaggagatgcgaaaactcttcatccagag
ggccaagaagatcgacaaaaataccccgattggcttccccatggccttccctcattttcaacatgcttctactggatca
tctacaagattgtccgtagagaggagcgtccacaaccagtga
```

### Ubc (from pLV-TRE-Sox17-Ubc-Bla):

```
Gagtgaacctctcagacagccacggagccatttccctccgtgggtgctccgacgcctagctcagcgggtctactattgcaac
taccocgacatcttgaggccgcctcggccattaattaagt , gatctggcctccgcgcgggttttggcgcctcccgcg
ggcgcceccctcctcagggcagcgtgccaactcagacgaagggcgcaggagcgtcctgataccttccgcccggacg
ctcaggacagcggcccgcctgctcataagactcggccttagaaccccagtatcagcagaaggacatctttaggacggga
cttgggtgactctagggcactggttttcttccagagagcggaaacagggcaggaaaagtagtcccttctcggcgatt
ctgcccagggatctccgtggggcggtgaaacccgatgattatataaggacgcgcgggtgtggcacagctagtctccg
tcgcagcggggaattgggtcgcgggtcttggttgtggaatcgtctgtaatcgtcacttgggtgagtagcgggctgctggg
ctggcgggggcttctgctggcgcgggcccgcctcgggtgggacggaagcgtgtggagagaccgccaagggtgtagtct
gggtccgcgagcaaggttgccctgaaactgggggttggggggagcgcagcaaaaatggcggctgttcccagctcttgaa
tggaaagacgcttgtgaggcgggctgtgaggtcgttgaaacaaggtggggggcattgggtgggcccgaagaacccaaggt
cttgaggccttccgtaaatgcccggaaagctcttattccgggtgagatgggctggggcaccaatcggggacctgacgtg
aagtttgtcactgactggagaactcggtttctgctctgttgcggggggcggcagttatgcccgttgggctgggacgtgc
accgctaccttggggagcgcgcgcctcgtcgtgtcgtgacgtcaccgcttctgttggcttataaatgcagggtgggg
ccacctgcccggtaggtgtgcccgttaggcttttctccgtcgcaggacgcagggttccgggcttagggtaggctctcctga
```

atcgacagggcgcggacctctgggtgaggggagggataagtgagggcgtcagtttctttgggtcggttttatgtacctat  
cttcttaagtagctgaagctccggttttgaaactatgctgctcggggttggcgagtggtgtttgtgaagtttttaggc  
acctttgaaatgtaatcatttgggtcaatatgtaattttcagtggttagactagtaaattgtccgctaaattctggc  
cgtttttggttttttggtagacgaag, cgtgagctcgaggctgactctagatgccaccatggccaagcctttgtct  
caaga

### Neo (from pcDNA3.1(+)):

Gggagcttgatataatccatcttccggatctgatcaagagacaggatgaggatcgtttcgc, atgattgaacaagatgga  
ttgacagcaggttctccggccgcttgggtggagaggctattcggctatgactgggcacaacagacaatccggctgctc  
tgatgccgcctgttccggctgtcagcgcaggggcccgggttctttttgtcaagaccgacctgtccgggtgccctga  
atgaactgcaggacgaggcagcgcggctatcgtggctggccacgacgggcttccctgctcagctgtgctcgacgctt  
gtcactgaagcgggaagggactggctgctattgggccaagtgccggggcaggatctcctgtcatctcaccctgtctcc  
tgccgagaaagtatccatcatggctgatgcaatgctggcggctgcatacgttggatccggctacctgcccattcgacc  
accaagcgaaacatcgcatcgagcgcagcgtactcggatggaagccggctcttctgctgacaggaatgctggacgaa  
gagcatcaggggctcgcgccagccgaactgttccgccaggctcaaggcgcgcgatgcccgacggcgaggatctcgtcgt  
gaccatggcgaatgcttggcgaataatcattgggtggaaaatggccgctttctggattcatcgactgtggccggc  
tgggtgtggcggaccgctatcaggacatagcgttggctaccgctgataattgctgaagagcttggcggcgaaatgggct  
gaccgcttctcgtgctttacgggtatcgccgctcccgattcgcagcgcacatcgcttctatcgccttcttggacgagtt  
cttctga, gccgggactctggggttcgaaatgaccgaccaagcgcagcggccaacctgcccattcagcag

### GLRA1 forward primer (GLRA1\_fwd\_EcoRI\_kzk\_2008-07-30):

Cgtagggaattcggccaccatgtacagcttcaatactcttcgactctacct

### GLRA1 reverse primer (GLRA1\_rev\_SfiI\_UbcOvlp\_2008-07-30):

gtagagaggacgtccacaaccagtgattggccgcctcggcctt

Reverse Compliment: blah

### Ubc forward primer (Ubc\_fwd\_GLRA1Ovlp\_SfiI\_2008-07-30):

cagtgattggccgcctcggccttctcctcgggtgtccgacgc

### Ubc reverse primer (Ubc\_rev\_kzk\_BleoOvlp\_2008-07-28):(works for Neo too)

ccgtttttGgcttttttggtagacgaaggc

### Neo forward primer (Neo\_fwd\_UbcOvlp\_2008-07-30):

gcttttttggtagacgaagggccaccatgattgaacaagatggattgcacgc

### Neo reverse primer (Neo\_rev\_BsrGI\_2008-07-30):

ctatcgccttcttggacgagttcttctgacgtacgatccataag

Reverse Compliment: blah

### Final Construct:

Cgtagggaattcggcaccatgtacagcttcaatactcttcgactctacctttgggagaccattgtattcttcagcct  
tgetgcttctaaggaggctgaagctgctcgcctccgcacccaagcctatgtcaccctcggatttccctggataagctaa  
tggggagaacctccggatgatgcccaggatcaggcccaattttaaaggctccccagtgaaactgagctgcaacatt  
ttcatcaacagcttttggttccattgctgagacaaccaatggactatagggccaacatcttccctgcccagcaatggaa  
cgacccccgcctggcctataatgaataccctgacgactctctggacctggacccatccatgctggactccatctgga  
aacctgacctgttctttgccaacgagaagggggccacttccatgagatcaccacagacaacaaattgctaaggatc  
tcccggaatgggaatgtcctctacagcatcagaatcaccctgacactggcctgccccatggacttgaagaatttccc  
catggatgtccagacatgtatcatgcaactggaaagctttggatatacagatgaatgacctcatctttgagtggcagg  
aacagggagccgtgaggtgagcagatggactaacctctgccccagtttatacttgaaggaagagaaggacttgagatac  
tgcaccaagcactacaacacaggtaaaattcacctgcattgaggcccggttccacctggagcggcagatgggttacta  
cctgattcagatgtatattcccagcctgctcattgtcatcctctcatggatctcctcttggatcaacatggatgctg  
cacctgctcgtgtgggcttaggcataccactgtgctcaccatgaccaccagagctccggctctcagacatctctg  
cccaagggtgctcctatgtgaaagccattgacatttggatggcagtttgcctgctctttgtgttctcagccctattaga  
ataatgctgcccgttaactttgtgtctcggcaacataaggagctgctccgattcaggaggaagcggagacatcaacagg  
aggatgaagctggagaaggccgctttaacttctctgctatgggatgggcccagcctgctacagggccaaggatggc  
atctcagtcaggggcgccaacaacagtaacaccaccaacccccctcctgcacatctaaagccccagaggagatggc  
aaaactcttcatccagaggggccaagaagatcgacaaaataccccgcatggcttccccatggccttctcattttca  
acatgttctactggatcatctacaagattgtccgtagagaggagctccacaaccagtgattggccgctcggccttc  
ctccgtgggtgctccgacgcctagctcagcgggtctactattgcaactaccccagacatttgaggccgctcggccattaat  
taagtgatctggcctccgcgcccgggttttggcgcctcccgcggggcgccccctcctcagggcgagcgtgccacgctc  
agacgaagggcgagggagcgtcctgacccctccgcccggacgctcaggacagcggcccgcctgctcataagactcggc  
cttagaaccacagtatcagcagaaggacattttaggacgggacttgggtgactctagggcactggttttcttccag  
agagcggaaacaggcgaggaaaagtagtcccttctcggcgattctgcccggaggatctccgtggggcggtgaaacgcga  
tgattataaaggacgcgcccgggtgtggcacagctagttccgtcgcagcccgggatttgggtcgcgggtctctgtttgt  
ggatcgtctgtgacgctcacttgggtgagtagcgggctgctgggctggcggggcttctcgtggcgcggggcgcctcgg  
tgggacggaaagcgtgtggagagaccgccaagggctgtagctcgggtccgcgagcaagggttgcctgaaactgggggtt  
ggggggagcgcagcaaaaatggcggctgttcccagctcttgaaatggaaagacgcttgtgagggcgggctgtgaggtcgtt  
gaaacaaggtggggggcatgggtgggcccgaagaaccaaggtcttgaggccttcgctaatgcccggaaagctcttatt  
cgggtgagatgggctggggcaccatctggggacccctgacgtgaagtttgtcactgactggagaactcggtttctcgt  
ctgttgcggggggcggcagttatgcccgtgcggttgggcagtgaccccgtaccttgggagcgcgcgcccctcgtcgtgt  
cgtgacgctcaccgctctgttggcttataatgcaggggtggggccacctgcggtaggtgtgcccgtaggctttctcc  
gtcgcaggacgcaggggttccggcctagggtaggctctcctgaaatcgacagggcgcggacccctgggtgaggggagggga  
taagtgagggcgtcagtttcttggctgggttttatagtacctatcttcttaagtagctgaagctccggttttgaaactat  
gcctcgggggttggcgagtggttttgtgaagtttttaggcaccttttgaatgtaatcatttgggtcaatatgta  
atcttcagtgtagactagtaaatgtccgctaaattctggccgtttttggcttttttggtagacgaaggccaccat  
gattgaaacaagatggattgacagcaggttctccggcgccttgggtggagaggctattccggctatgactgggcacaac  
agacaaatccgctgctctgatgcccgcgctgttccggctgtcagcgcagggggcgcggcttctttttgtcaagaccgac  
ctgtccgggtgccctgaaatgaaactgcaggacgagggcagcgcggctatcgtggctggccacgacgggcttccctgccc  
agctgtgctcagcgttgtcactgaagcgggaagggactggctgctatggggcgaagtgcgggggaggaatcctcgt  
catctcaccttgcctcctgcccagaaaagtatccatcatggctgatgcaatgcggcggctgcatacgttgaatccggct  
acctgccatttcgaccaccaagcgaacaatcgcatcgagcgcagcagctactcggatggaagccggctctgtcgaatca  
ggatgatctggacgaagagcatcaggggctcgcgccagccgaactgttcgccaggctcaaggcgcgcatgcccagcgc  
gagaggatctcgtcgtgacccaatggcgaatgctgcttgcgcaatatacgtgggaaatggccgctttctggatctc  
atcagactgtggccggctgggtgtggcggaccgctatcaggacatagcgttggctaccgctgataatgctgaagagct  
tggcggcgaatgggctgaccgcttctcgtgctttacggtaatcgcgcctcccgaatcgcagcgcatacgccttctatac  
gccttcttgacgagttctcttgacgtacgatccataag