

## pLV-Ubc-rtTA-2A-LacIKrab-IRES-Puro

**Backbone:** pLV-Ubc\_XbaI\_rtTA-2A-LacI/Krab-IRES2-Bla\_ClaI

**OR** pLV-Ubc\_XbaI\_XbaI\_ClaI\_Puro (**Choose either**)

**Insert:** XbaI\_rtTA-2A-LacIKrab-IRES2-Puro\_ClaI

## Parts:

### rtTA (from pLV-Ubc-rtTA-SecPen-IRES2-mCherrym):

```
ctggcggctttttggcctttt ttgtagacgaagcgtgagctcgagg tgcactctagaggatccccgggt accggaggc  
cattacggcct cgcgccaccatgtctagattagataaaaag taaagtgattaacagcgcattagagctgcttaatgagg  
tcggaatcgaaggtttaacaacccgtaaaactcgcccagaagcttgggtgtagagcagcctacactgtattggcatgta  
aaaaataagcgggctttgctcgacgccttagccattgagatgtagataggcaccatactcacttttgccctttaa  
aggggaaagctggcaagattttttacgcaataacgctaaaagtttagatgtgctttactaagtcacgcaatggag  
caaaagtacattcagatacacggcctacagaaaaacagtatgaaactctcgaaaatcaattagcctttttatgcca  
caaggtttttcactagagaacgcgcttatatgcactcagcgcgtgtggggcattttacttttaggttgcgatattggaaga  
tcaagagcatcaagtcgctaaagaagaaagggaaacacctaactactgatagtagtgcgcgcatattacgacaagcta  
tcgaattatgtgatcaccaaggtgcagagccagccttcttattcggccttgaattgatcatatgcggttagaaaa  
caacttaaagtgtgaaagtgggtccgcgtacagccgcgcgctacgaaaaacaattacgggtctaccatcgagggcct  
gctcgatctcccggacgacgacgcccccgaaagaggcggggctggcggtccgcgcctgtcctttctcccgcgggac  
acacgcgcagactgtcgacggcccccccgaccgatgtcagcctgggggacgagctccacttagacggcgaggacgtg  
gcgatggcgcatgcccagcgcgctagacgatttcgatctggacatgttgggggacggggattccccgggtccgggatt  
tccccccacgactccgccccctacggcgcctctggatattggccgacttcgagtttgagcagatgtttaccgatgccc  
ttggaa ttgacgagtagcgggtgggtat
```

### LacIKrab-IRES2 (from pLV-CMVTO-LacIKRAB-IRES2-EGFP):

```
atgaaaccagtaacggtatacgatg tgcgagagtagtgcgggtgtctcttatcagaccggtttcccgcgtgggtgaacca  
ggccagccacgctttctgcgaaaacgcgggaaaaagtggaagcggcgatggcgggagctgaattacattcccaccgcg  
tggcacaacaactggcgggcaaacagtcggttgcgtgattggcggttggccacctccagtcctggcctgcacgcgcgctcg  
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cgtcgaagcctgtaaagcggcggtgcacaatcttctcgcgcaacgcgctcagtgggctgatcattaactatccgctgg  
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cccatcaacagatattatctcccatgaagacggtagcgcgactggcggtggagcatctggctcgcatgggtcacca  
gcaaactcgcgctgttagcgggcccattagttctgtctcggcgcgctctgcgctctggctggctggcataaatactca  
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taccgagtcggggctgcgcgcttgggtgcggatatactcggtagtgggatacagacgataccgaagacagctcatgttata  
tcccgcggttaaccaccatcaaacaggattttcgcctgctggggcaaacacagcgtggaccgcttggctgcaactctct  
cagggccaggcgggtgaagggcaatcagctgttgcgcgctctcactggtgaaaagaaaaaccacctggcgcccaatac  
gcaaaccgcctctcccgcgcgcttggccgattcattaatgcagctggcagcagaggtttcccgactggaaagcggggc
```

agagaccgccccaaaaagaagagaaaggtcgacggcggtggtgctttgtctcctcagcactctgctgtcactcaagga  
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cgtctaggccccccgaaccacggggacgtgggtttcctttgaaaaacacgatgataaatatggccaca

### **Puromycin (from pLV-Ubc-Puro):**

Gccaccatgaccgagtacaagcccacgggtgcgctcgccaccgcgacgacgtccccggggcgtacgcaccctcgc  
cgccgcttcgcccactaccccgccacgcgccacaccgtcgaccggaccgccacatcgagcgggtcaccgagctgc  
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tcgatatcaagcttatcgataatcaacctctggattacaaaatttgtgaaagattgactgg

### **rtTA-2A-LacIKrab-IRES2 forward primer (rtTA-2A-LacIKrab-IRES\_fwd\_XbaI\_tcc\_2008-07-16):**

atgcatctagatcgcccaccatgtccagattagataaaaag

### **rtTA-2A reverse primer (rtTA-2A\_rev\_LacIKrOverlap\_2008-07-14):**

ttgacgagtagcgggtgggtatgccaccaacttctctctgctgaagcagggc

### **LacIKrab forward primer (LacIKrab\_fwd\_rtTA2AOverlap\_2008-07-14):**

tgaagcaggccggcgacgtggaggagaaccaggcccaatgaaaccagtaacgttatacgtg

### **rtTA-2A-LacIKrab-IRES2 reverse primer (rtTA-2A-LacIKrab-IRES\_rev\_PuroOvlp\_2008-07-16):**

cctttgaaaaacacgatgataaatatggccacagccaccatgaccgagt

### **Puro forward primer (Puro\_fwd\_IRESOvlp\_2008-07-16):**

gataaatatggccacagccaccatgaccgagtacaagcccac

### **Puro reverse primer (Puro\_rev\_XbaIRmvd\_ClaI\_2008-07-21):**

gcccgggtgccatgatacaagagaattcatogataatcaac

## Final Construct:

atgcatctagatcgccaccatgtccagattagataaaaagtaaagtgattaacagcgcattagagctgcttaatgag  
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tcgataatcaac