

## Plasmid List

Reference:

***Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system***

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2. **CMV-Brainbow-1.0 "H"**: tdimer2 ; M-mEYFP ; M-mCerulean.
3. **CMV-Brainbow-1.0 "L"**: dTomato ; EYFP ; mCerulean.
4. **CMV-Brainbow-1.1 "M"**: Kusabira ; M-mCherry ; M-mEYFP ; M-mCerulean.
6. **CMV-Brainbow-2.1 "R"**: hrGFPII-NLS; EYFP ; tdimer2 ; M-mCerulean.
8. **Thy1-Brainbow-1.0 "H"**: tdimer2 ; M-mEYFP ; M-mCerulean.
9. **Thy1-Brainbow-1.0 "L"**: dTomato ; EYFP ; mCerulean
10. **Thy1-Brainbow-1.1 "M"**: Kusabira ; M-mCherry ; M-mEYFP ; M-mCerulean.
12. **Thy1-Brainbow-2.1 "R"**: hrGFPII-NLS; EYFP ; tdimer2 ; M-mCerulean.

- Some constructs have cytoplasmic proteins or membrane tethered fluorescent proteins (indicated as "M-XFP" in the list below). We found that cytoplasmic fluorescent proteins give a nice labeling of neuronal cell bodies and large processes, while membrane fluorescent proteins can help visualizing very fine axonal processes. One construct (#3 CMV-Brainbow-1.0 "L") encodes only cytoplasmic proteins, and is recommended for most applications, while other constructs usually encode some membrane-bound proteins.

- Constructs are available either with a Thy1 or CMV promoter. We recommend that you would use the CMV-Brainbow plasmids if you want to excise the Brainbow cassette and put it in another vector.

- Brainbow-1.0 and Brainbow-1.1 construct are compatible with any Cre driver, and are therefore more flexible than Brainbow-2.0 and Brainbow-2.1 which function using transient Cre activity (for instance CreER + tamoxifen injection).

- Multichannel fluorescent imaging of Brainbow-1.0 is very straightforward. With Brainbow-1.1 and Brainbow-2.1, spectrally separating all 4 fluorescent proteins may require linear unmixing.

#2 - CMV-Brainbow-1.0 "H" [TYC]

CMV-Lox2272-LoxP-tdimer2-bGHpA-Lox2272-MbmEYFP-SV40pA-LoxP-MbmCerulean-FRT-SV40pA

In p-EGFP-N1 (Clontech) vector backbone (KanR)

ATAACTTCGTATAGGATACTTTATACGAAGTTAT = Lox2272

ATAACTTCGTATAGCATAACATTATACGAAGTTAT = LoxP

GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC = FRT

**ATG = start of XFP ORFs**

Mb = palmitoylation tag

m = monomeric version of XFP

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGG  
AGTTCCGCGTTACATAACTTACGGTAAATGGCCCCGCCTGGCTGACCGCCCAA  
CGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA  
ATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCC  
ACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTC  
AATGACGGTAAATGGCCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGG  
ACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTG  
ATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGG  
GATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAA  
TGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGT  
GAACCGTCAGATCCGCTAGCGAGCTCATAACTTCGTATAGGATACTTTATACG  
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CAAAGAGTTCATGCGCTTCAAGGTGCGCATGGAGGGCTCCGTGAACGGCCAC  
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ACCGCCAAGCTGAAGGTGACCAAGGGCGGCCCTGCCCTTCGCCTGGGACA  
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CGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCT  
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CGCGGCTAGATAATAGGGTACCACCTTTAGTGAGGGTTAATTGCTAGAGCTC  
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CACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACT  
TCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTA  
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TGTC AAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCG  
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ACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCC  
CGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGG  
AGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCT  
GTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGG  
GGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTG  
GCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCT  
GTGGATAACCGTATTACCGCCATGCAT

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#3 - Sequence CMV-Brainbow-1.0 "L" [CytoBow]

CMV-Lox2272-LoxP-dTomato-SV40pA-Lox2272-mCerulean-SV40pA-LoxP-EYFP-SV40pA

In p-EGFP-N1 (Clontech) vector backbone (KanR)

ATAACTTCGTATAGGATACTTTATACGAAGTTAT = Lox2272

ATAACTTCGTATAGCATAACATTATACGAAGTTAT = LoxP

(Note: no FRT site in this construct)

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGG  
AGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAA  
CGACCCCGCCCAATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA  
ATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCC  
ACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTC  
AATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGG  
ACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTG  
ATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTTGACTCACGGG  
GATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAA  
TGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGT  
GAACCGTCAGATCCGCTAGCGAGCTCATAACTTCGTATAGGATACTTTATAC  
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CGGCAGATCTGCCACCATGGTGAGCAAGGGCGAGGAGGTCATCAAAGAGTT  
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AGCAATAGCATCACAAATTCACAAATAAAGCATTTTTTTCACTGCATTCTAG  
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AATGCTCCGAGGGCCGCCACCACCTGTTCTGTAGGAATTGGCCGCGACTCT  
AGGATCTAGGATAACTTCGTATAGGATACTTTATACGAAGTTATTGATCCACC

GGTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTACCCGGGGTGGTGCC  
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GCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGG  
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TCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAATGCGCCGCTACAGGG  
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GGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGAT  
GCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGAC  
CGACCTGTCCGGTGCCCTGAATGAACTGCAAGACGAGGCAGCGCGGCTATCG  
TGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGA  
AGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTG  
TCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCG  
GCGGCTGCATACGCTTGATCCGGCTACCTGCCATTTCGACCACCAAGCGAAA  
CATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTGATCAGG  
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GCTCAAGGCGAGCATGCCCGACGGCGAGGATCTCGTCTGTGACCCATGGCGAT  
GCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGA  
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CGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGTGC  
TTTACGGTATCGCCGCTCCCGATTTCGAGCGCATCGCCTTCTATCGCCTTCTT  
GACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGAC  
GCCAACCTGCCATCACGAGATTTTCGATTCCACCGCCGCCTTCTATGAAAGGT  
TGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGG  
GGATCTCATGCTGGAGTTCTTCGCCACCCTAGGGGGAGGCTAACTGAAACA  
CGGAAGGAGACAATAACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAGA  
CAGAATAAAACGCACGGTGTGGGTCGTTTGTTCATAAACGCGGGGTTCCGGT  
CCCAGGGCTGGCACTCTGTGATACCCACCGAGACCCCATTTGGGGCCAATA  
CGCCCGCGTTTCTTCCTTTTCCCCACCCCAAGTTCGGGTGAAGGCC  
CAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCCTGCCATAGCCTCAGGTTA  
CTCATATATACTTTAGATTGATTTAAAACCTTCATTTTTAATTTAAAAGGATCT  
AGGTGAAGATCCTTTTTGATAATCTCATGACCAAATCCCTTAACGTGAGTTT  
TCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAG  
ATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCCGCTA  
CCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGT  
AACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCG  
TAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCT  
GCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCG  
GGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGGCTAAC  
GGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTG  
AGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGA



AAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACG  
AGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCTGGGTTTC  
GCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAG  
CCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCT  
GGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACC  
GTATTACCGCCATGCAT

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#4 - CMV-Brainbow-1.1 'M' [ORYC]

CMV-LoxN-Lox2272-LoxP-Kusabira-SV40pA-LoxN-MbmCherry-SV40pA-  
Lox2272-MbmEYFP-SV40pA-LoxP-MbmCerulean-FRT-SV40pA

In p-EGFP-N1 (Clontech) vector backbone (KanR)

ATAACTTCGTATAGTATACCTTATACGAAGTTAT = LoxN

ATAACTTCGTATAGGATACTTTATACGAAGTTAT = Lox2272

ATAACTTCGTATAGCATACATTATACGAAGTTAT = LoxP

GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC = FRT

**ATG = start of XFP ORFs**

Mb= palmitoylation tag

mXFP = monomeric version of XFP

Note: the version of Kusabira Orange (KO) used here is the 'non-monomeric' one

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TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGG  
AGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAA  
CGACCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA  
ATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCC  
ACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTC  
AATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGG  
ACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTG  
ATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGG  
GATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGGTGGCACCAA  
AATCAACGGGACTTTCCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAA  
TGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTAGT  
GAACCGTCAGATCCGCTAGCTCGAGATAACTTCGTATAGTATACCTTATACG  
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CGAGATAACTTCGTATAGCATACATTATACGAAGTTATACCGGCAGATCTCG  
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CGGCTCCGTCAATGGGCATGAGTTCACAGTTGAAGGTGAAGGCACAGGCAAA  
CCTTACGAGGGACATCAAGAGATGACACTACGCGTCACAATGGCCAAGGGC  
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TAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCATCACAA  
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CTCATCAATGTATCATAACTTCGTATAGTATACCTTATACGAAGTTATAGGAT  
CTCCGCCACCATGCTGTGCTGCATCAGAAGAATAAACCGGTTGAGAAGAAT  
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CCGGGATCCACCGGTCGCCACCATGGTGAGCAAGGGCGAGGAGCTGTTACC  
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TGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAA  
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CTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCACCCTAGGGGGAGGC  
TAACTGAAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGG  
CAATAAAAAGACAGAATAAAACGCACGGTGTGGGTCGTTTGTTCATAAACG  
CGGGGTTCCGGTCCAGGGCTGGCACTCTGTGATACCCACCGAGACCCCAT  
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GTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGG  
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ACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCC  
CGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGG  
AGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCT  
GTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGG  
GGGGCGGAGCCTATGGAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTG  
GCCTTTTGCTGGCCTTTTGCTCACATGTTCTTCTGCGTTATCCCCTGATTCT  
GTGGATAACCGTATTACCGCCATGCAT

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#6 - CMV-Brainbow-2.1 "R" [GYRC]

CMV-LoxP-hrGFPIINLS-bGHpA-SV40pA-EYFP-LoxP-LoxP-tdimer2-bGHpA-SV40pA-MbmCerulean-LoxP-FRT-SV40pA

(in italic: sequences in reverse orientation)

In p-EGFP-N1 (Clontech) vector backbone (KanR)

ATAACTTCGTATAGCATAACATTATACGAAGTTAT = LoxP

ATAACTTCGTATAATGTATGCTATACGAAGTTAT = LoxP reverse oriented

GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTC = FRT

**ATG / CAT= start of XFP ORFs**

Mb = palmitoylation tag

NLS =nuclear localization signal

mXFP = monomeric version of XFP

TAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGG  
AGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAA  
CGACCCCCGCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCA  
ATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCC  
ACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTC  
AATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGG  
ACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTG  
ATGCGGTTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGG  
GATTTCCAAGTCTCCACCCATTGACGTCAATGGGAGTTTGTGGCACCAA  
AATCAACGGGACTTTCAAAATGTCGTAACAACCTCCGCCCCATTGACGCAA  
TGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTGGTTTTAGT  
GAACCGTCAGATCCGCTAGCGCTACCGGACTCAGATCGAGATAACTTCGTAT  
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AGGGCAACATCCTGTTTCGGCAACCAGCTGGTGCAGATCCGCGTGACCAAGGG  
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ACCGCACCTTACCAAGTACCCCGAGGACATCAGCGACTTCTTCATCCAGAG  
CTTCCCCGCCGGCTTCGTGTACGAGCGCACCCCTGCGCTACGAGGACGGCGGC  
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CGTGCTGGTGGGCCAGGTGATCCTGGTGTACCGCCTGAACAGCGGCAAGTTC  
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CGGCGGCTTCGTAGAGCAGCACGAGACCGCCATCGCCCAGCTGACCAGCCTG  
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CTCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAG  
GTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGAT  
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TATAGGAACTTCTCGAAGCTTGAAGCTCGAGATCTGAGTCCGGTAGCGCTAGTT  
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CATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCAT  
GCAT

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## Sequence of the Thy1 genomic element or 'promoter'

**Sequence from sequencing of extremities + NCBI database; might not be 100% accurate**

In pUC18 vector (AmpR)

Inserts are cloned in the Xho I site. No polyadenylation sequence is needed

To linearize plasmid and remove vector sequences, EcoRI + PvuI , or alternatively NotI + PvuI are used (Note that PvuI also cuts inside the pUC18 vector)

GACGAAAGGGCCTCGTG...: pUC18 sequence in 5' and 3'

TCTGAGTGGCAAAGGACCTTAGG: linker in between PUC18 sequence and Thy1 genomic sequence

AATTCAGAGACCGGG...: Thy1 genomic sequence

**TCTGAGTGGCAAAGGACCTTAGG: Thy1 F1 primer**

**GGTCAGCTGACTCCCTCCCAAGTC: ThyR1 primer**

**CTCGAG: Xho site in between**

**Thy1 vector complete sequence:**

GACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATA  
ATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCC  
CTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAAT  
AACCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCA  
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GCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTG  
CACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAG  
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