

pMSCV-Hyg-GFP-miR Cntl

Absent Sites	0	AarI, AbsI, Accl, Alfl, Alfl', Apal, AvrII, BamHI, BarI, BarI', BbsI, BclI, BplI, BplI', BsaAI, BsaBI, BsiWI, BstBI, BstZ17I, CspCI, CspCI', FseI, FspAI, HpaI, MauBI, MfeI, MluI, MreI, NruI, PacI, PflMI, PmeI, PmlI, PstI, PspOMI, PspXI, PstI, PstI', Sall, SanDI, SbfI, SfiI, SgrDI, SnaBI, SrfI, SwaI, XcmI, XhoI
AfIII	1	4810
AjuI	1	3422
AjuI'	1	3390
AsiSI	1	3073
BglIII	1	1411
BplI	1	2456
BsmI	1	2614
BstEII	1	1089
BstXI	1	2699
Clal	1	3741
HincII	1	3545
HindIII	1	2694
NotI	1	2158
NsiI	1	3740
PciI	1	4810
PshAI	1	2736
RsrII	1	3117
SacII	1	3489
SexAI	1	1217
SgrAI	1	7246
StuI	1	2477

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5' TGAAAGACCCACCTGTAGGTTTGGCAAGCTAGCTTAAGTAACGCCATTTTGAAGGCATGGAAAATACATAACTGAGAATAGAGAAGTTTCAAGTCAAGG
 100
 3' ACTTTCTGGGTGGACATCCAAACCGTTCGATCGAATTCATTGCGGTAACCGTTCCTGACCTTTTATGTATTGACTCTTATCTCTTCAAGTCTAGTTCC
 5' pCMV LTR

5' TTAGGAACAGAGAGACAGCAGAATATGGGCCAAACAGGATATCTGTGGTAAGCAGTTCTGCCCCGGCTCAGGGCCAAGAACAGATGGTCCCCAGATGCG
 200
 3' AATCCTTGTCTCTCTGTGCTTATACCCGGTTTGTCTTATAGACACCATTTCGTCAAGGACGGGGCCGAGTCCCGGTTCTTGTCTACCAGGGGTCTACGC
 5' pCMV LTR

5' GTCCCGCCCTCAGCAGTTTCTAGAGAACCATCAGATGTTTCCAGGGTGCCCCAAGGACCTGAAATGACCCTGTGCCTTATTTGAACTAACCAATCAGTTC
 300
 3' CAGGGCCGGGAGTCGTCAAAGATCTCTTGGTAGTCTACAAAGTCCACGGGGTTCCTGGACTTTACTGGGACACGGAATAAACTTGATTGGTTAGTCAAG
 5' pCMV LTR

5' GCTTCTCGTCTCTGTTCGCGCCTTCTGCTCCCCGAGCTCAATAAAAAGAGCCACAAACCCCTCACTCGGCGCGCAGTCTCCGATAGACTGCGTCCCC
 400
 3' CGAAGAGCGAAGACAAGCGCGGAAGACGAGGGGCTCGAGTTATTTTCTCGGGTGTGGGGAGTGAGCCGCGCGGTGAGGAGGCTATCTGACGCAGCGGG
 5' pCMV LTR

5' GGGTACCCGTATTCCCAATAAAGCCTCTTGCTGTTTGCATCCGAATCGTGGACTCGCTGATCCTTGGGAGGGTCTCCTCAGATTGATTGACTGCCACCT
 500
 3' CCCATGGGCATAAGGGTTATTTTCGGAGAACGACAAACGTAGGCTTAGCACCTGAGCGACTAGGAACCCCTCCAGAGGAGTCTAACTAACTGACGGGTGGA
 5' pCMV LTR

5' CGGGGTCTTTTCAATTTGGAGGTTCCACCGAGATTGGAGACCCCTGCCAGGGACCACCGACCCCCCGCGGGAGGTAAGCTGGCCAGCGGTCTGTTTCG
 600
 3' GCCCCAGAAAGTAAACCTCCAAGGTGGCTCTAAACCTCTGGGGACGGGTCCCTGTTGGCTGGGGGGCGGCCCTCCATTCGACCGGTGCGCAGCAAAGC
 5' pCMV LTR

Pack Signal

5' TGTCTGTCTCTGTCTTGTGCGTGTGTGTGCCGGCATCTAATGTTTGGCCCTGCGTCTGTACTAGTTAGCTAACTAGCTCTGTATCTGGCGGACCCGTGG
 700
 3' ACAGACAGAGACAGAAACACGCACAAACACGGCCGTAGATTACAAACCGCGACGCAGACATGATCAATCGATTGATCGAGACATAGACCGCTGGGCACC
 Pack Signal

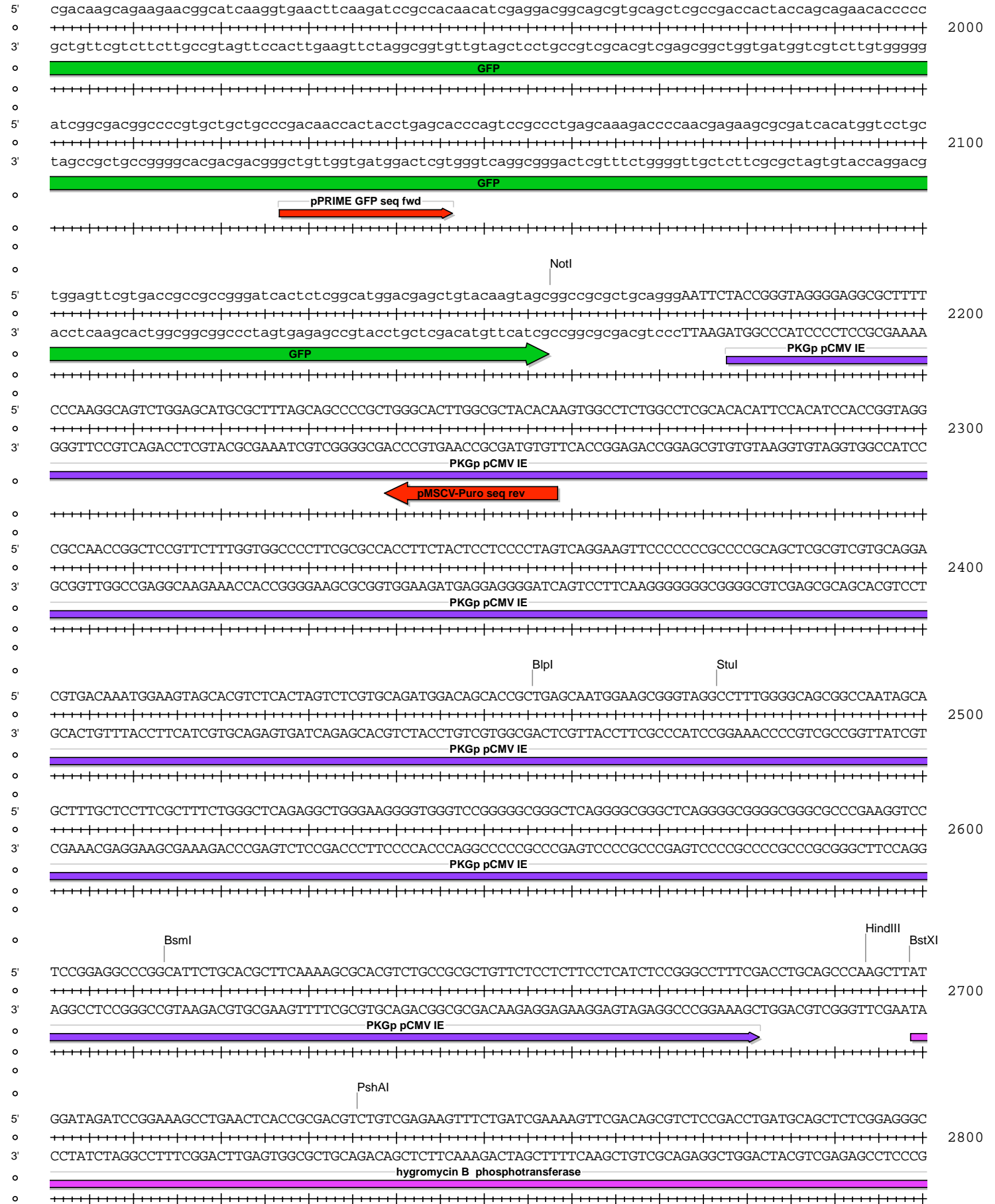
5' TGGAATGACGAGTTCTGAACACCCGGCCGCAACCTGGGAGACGTCCCAGGGACTTTGGGGCCGTTTGTGGCCCGACCTGAGGAAGGGAGTCGATG
 800
 3' ACCTTGACTGCTCAAGACTTGTGGGCCGGCGTTGGGACCTCTGCAGGGTCCCTGAAACCCCGGCAAAAACACCGGGCTGGACTCCTTCCTCAGCTAC
 Pack Signal

5' TGGAATCCGACCCCGTCAGGATATGTGGTTCTGGTAGGAGACGAGAACC'TAAACAGTTCCCGCCTCCGTCTGAATTTTGTCTTTCGGTTTGAACCGAA
 900
 3' ACCTTAGGCTGGGGCAGTCTTATACACCAAGACCATCCTCTGCTCTTGGATTTTGTCAAGGGCGGAGGCAGACTTAAAAACGAAAGCCAAACCTTGGCTT
 Pack Signal

5' GCCGCGCTCTGTCTGCTGCAGCGCTGCAGCATCGTTCGTGTGTCTCTGTCTGACTGTGTTTCTGTATTTGTCTGAAAATTAGGGCCAGACTGTTAC
 1000
 3' CGGGCGCAGAACAGACGACGTCGCGACGTCGTAGCAAGACACAAACAGAGACAGACTGACACAAAGACATAAACAGACTTTTAATCCCGGTCTGACAATG
 Pack Signal



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5' GAAGAATCTCGTGCTTTCAGCTTCGATGTAGGAGGGCGTGGATATGTCCGCGGGTAAATAGCTGCGCCGATGGTTTCTACAAAGATCGTTATGTTTATC 2900
 +-----+
 3' CTTCTTAGAGCACGAAAGTCGAAGCTACATCCTCCCGCACCTATACAGGACGCCATTTATCGACGCGGCTACCAAAGATGTTTCTAGCAATACAAATAG
 hygromycin B phosphotransferase

5' GGCAC TTTGCATCGGCGCGCTCCCGATTCCGGAAGTGCFTGACATTTGGGAATTACAGCAGAGCCTGACCTATGCATCTCCCGCGTGACAGGGTGT 3000
 +-----+
 3' CCGTGAAACGTAGCCGGCGGAGGGCTAAGGCCTTACGAACTGTAACCCCTTAAGTCGCTCTCGGACTGGATAACGTAGAGGGCGGCACGTGTCCACA
 hygromycin B phosphotransferase

5' CACGTTGCAAGACCTGCCTGAAACCGAACTGCCCGCTGTCTGACGCGGCTCGCGGAGGCCATGGATGCGATCGCTGCGGCCGATCTTAGCCAGACGAGC 3100
 +-----+
 3' GTGCAACGTTCTGGACGGACTTTGGCTTGACGGGCGACAAGAGCTCGGCCAGCGCTCCGGTACCTACGCTAGCGACGCCGGCTAGAATCGGTCTGCTCG
 hygromycin B phosphotransferase

5' GGGTTCGGCCATTTCGGACCGCAAGGAATCGGTCAATACACTACATGGCGTGATTTTCATATGCGCGATTGCTGATCCCCATGTGTATCACTGGCAAACCTG 3200
 +-----+
 3' CCCAAGCCGGTAAGCCTGGCGTTCCCTTAGCCAGTTATGTGATGTACCGCACTAAAGTATACGCGCTAACGACTAGGGGTACACATAGTGACCGTTTGGAC
 hygromycin B phosphotransferase

5' TGATGGACGACACCGTCAGTGCCTCCGTCGCGCAGGCTCTCGATGAGCTGATGCTTTGGGCCGAGGACTGCCCGAAGTCCGGCACCTCGTGACCGCGGA 3300
 +-----+
 3' ACTACCTGCTGTGGCAGTCACGCAGGCAGCGCTCCGAGAGCTACTCGACTACGAAACCGGCTCCTGACGGGGCTTCAGGCCGTGGAGCACGTGCGCCT
 hygromycin B phosphotransferase

5' TTTCGGCTCCAACAATGTCTGACGGACAATGGCCGCATAACAGCGGTCAATTGACTGGAGCGAGGCGATGTTTCGGGGATTCCAATACGAGGTCGCCAAC 3400
 +-----+
 3' AAAGCCGAGGTTGTTACAGGACTGCCTGTTACCGCGTATTGTGCGCCAGTAACTGACCTCGCTCCGCTACAAGCCCCTAAGGGTTATGCTCCAGCGGTTG
 hygromycin B phosphotransferase

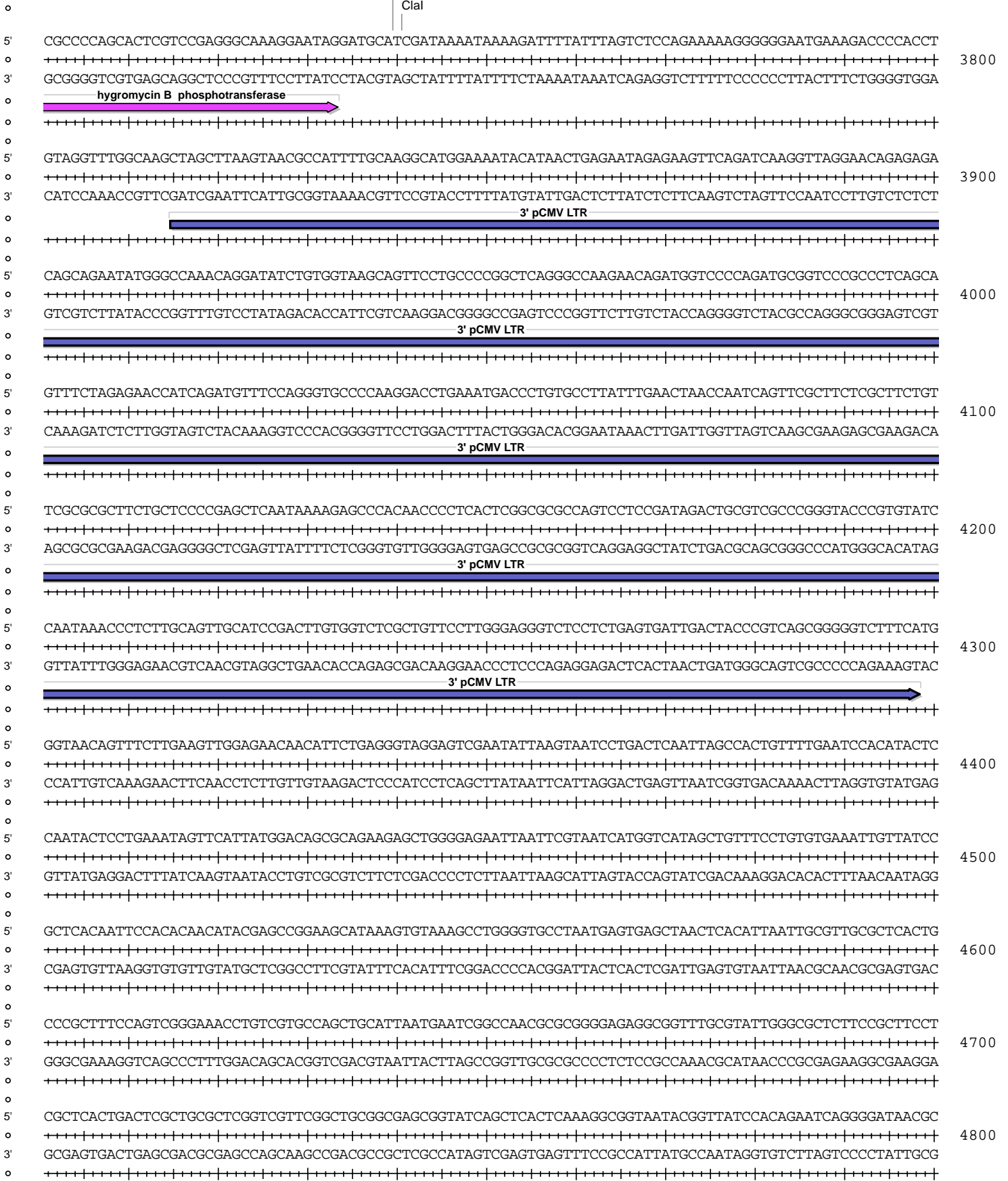
5' ATCTTCTTCTGGAGGCCGTGGTTGGCTTGATGGAGCAGCAGACGCGCTACTTCGAGCGGAGGCATCCGGAGCTGCAGGATCGCCGCGGCTCCGGGCGT 3500
 +-----+
 3' TAGAAGAAGACCTCCGGCACCAACCGAACATACCTCGTCTGCGCGATGAAGCTCGCCTCCGTAGGCTCGAACGCTCCTAGCGGCGCCGAGGCCCGCA
 hygromycin B phosphotransferase

5' ATATGCTCCGCATTGGTCTTGACCAACTCTATCAGAGCTTGGTTGACGGCAATTTTCGATGATGCAGCTTGGGCGCAGGGTTCGATGCGACGCAATCGTCCG 3600
 +-----+
 3' TATACGAGGCGTAACCAGAACTGGTTGAGATAGTCTCGAACCAACTGCCGTTAAAGCTACTACGTCGAACCCGCGTCCCAGCTACGCTGCGTTAGCAGGC
 hygromycin B phosphotransferase

5' ATCCGAGCCGGGACTGTGCGGCGTACACAAATCGCCCGCAGAAGCGCGCGCTCTGGACCGATGGCTGTGTAGAAGTACTCGCCGATAGTGGAACCGA 3700
 +-----+
 3' TAGGCTCGGCCCTGACAGCCCGCATGTGTTTAGCGGGCGTCTTCGCGCCGGCAGACCTGGCTACCGACACATCTTCATGAGCGGCTATCACCTTTGGCT
 hygromycin B phosphotransferase

pEHyg miR fwd seq primer

NsiI
Clal



pMSCV-Hyg-GFP-miR Cntl

Pcil
AflIII

5' AGGAAAGAACATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCAT
 4900
 3' TCCTTTCCTGTACACTCGTTTTCCGGTCTGTTTTCCGGTCTTGGCATTTTTCCGGCGCAACGACCGCAAAAAGGTATCCGAGGCGGGGGACTGCTCGTA

5' CACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTC
 5000
 3' GTGTTTTTAGTGCAGGTTTCAGTCTCCACCGCTTTGGGCTGTCTGATATTTCTATGGTCCGCAAAGGGGACCTTCGAGGGAGCACGCGAGAGGACAAG

5' CGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGT
 5100
 3' GCTGGGACGGCGAATGGCCTATGGACAGCGGAAAGAGGAAGCCCTTCGCACCGCAAAGAGTATCGAGTGCACATCCATAGAGTCAAGCCACATCCA

5' CGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGTTCAGCCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAAGTCCAACCCGTAAGACAC
 5200
 3' GCAAGCGAGTTTCGACCCGACACAGTGTCTGGGGGCAAGTCCGGCTGGCGACGCGGAATAGGCCATTGATAGCAGAACTCAGGTTGGGCCATTCTGTG

5' GACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGCT
 5300
 3' CTGAATAGCGGTGACCGTCTGTCGGTACCATTTGCTCTAATCGTCTCGCTCCATACATCCGCCAGATGTCTCAAGAACTTACCACCGGATTGATGCCGA

5' AACTAGAAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGG
 5400
 3' TGTGATCTTCTGTCAATAACCATAGACGCGAGACGACTTCGGTCAATGGAAGCCTTTTCTCAACCATCGAGAACTAGGCCGTTTGTGGTGGCGACC

5' TAGCGGTGGTTTTTTTGTGGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGG
 5500
 3' ATCGCCACCAAAAAACAAACGTTTCGTCTAATGCGCGTCTTTTTTCTAGAGTTCTTCTAGGAACTAGAAAAGATGCCCCAGACTGCGAGTCAAC

5' AACGAAACTCACGTTAAGGGATTTGGTTCATGAGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAAATGAAGTTTTAAATCAATCTAAA
 5600
 3' TTGCTTTTGGTGCATTCCTTAAACAGTACTTAATAGTTTTTCTAGAAAGTGGATCTAGGAAATTTAATTTTACTTCAAATTTAGTTAGATTT

5' GTATATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGCTATTTTCGTTTCATCCATAGTTGCCGACT
 5700
 3' CATATATACTCATTGAAACCAGACTGTCAATGGTTACGAATTAGTCACTCCGTGGATAGAGTCGCTAGACAGATAAAGCAAGTAGGTATCAACGGACTGA

Amp Res

5' CCCCCTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGGAGACCCACGCTCACCGGCTCCAGATTTATCA
 5800
 3' GGGGACGACATCTATTGATGCTATGCCCTCCCGAATGGTAGACCGGGTACGACGTTACTATGGCGCTCTGGGTGCGAGTGGCCGAGGTCTAAATAGT

Amp Res

5' GCAATAAACACGACCCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGAAGCTAGAGTAA
 5900
 3' CGTTATTTGGTCCGCTCCCGCTCCTTCCAGGACGTTGAAATAGCGGAGGTAGGTCAGATAATTAACAACGCCCTTCGATCTCATT

Amp Res

5' GTAGTTCGCCAGTTAATAGTTTGGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTTGGTATGGCTTCAATCAGCTCCGGTTC
 6000
 3' CATCAAGCGTCAATTTATCAAACGCGTTGCAACAACGGTAACGATGTCCGTAGCACACAGTGCAGAGCAGAAACCATAACGAAGTAAGTCCGAGGCCAAG

Amp Res

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5' CCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAAGCGTTAGCTCCTTCGGTCCCGATCGTTGTCAGAAGTAAGTTGGCCGAGTG
 6100
 3' GGTTGCTAGTTCGGCTCAATGTACTAGGGGGTACAACACGTTTTTTTCGCAATCGAGGAAGCCAGGAGGCTAGCAACAGTCTTCATTCAACCGGCGTCCAC
 Amp Res

5' TTATCACTCATGGTTATGGCAGCACTGCATAATCTCTTACTGTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCT
 6200
 3' AATAGTGAGTACCAATACCGTCGTGACGTATTAAGAGAATGACAGTACGGTAGGCATTCTACGAAAAGACACTGACCACCTCATGAGTTGGTTGAGTAAAGA
 Amp Res

5' GAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAA
 6300
 3' CTCCTTATCACATACGCCGCTGGCTCAACGAGAACGGGCCGAGTTATGCCCTATTATGGCGCGGTGTATCGTCTTGAAATTTTCACGAGTAGTAACCTTT
 Amp Res

5' ACGTCTTCGGGGCGAAAACCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCCACTGATCTTCAGCATCTTTTACT
 6400
 3' TGCAAGAAGCCCGCTTTTGAGAGTTCTTAGAATGGCGACAACCTAGGTCAGCTACATTGGGTGAGCACGTGGGTTGACTAGAAGTCGTAGAAAATGA
 Amp Res

5' TTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTTCTCTTT
 6500
 3' AAGTGGTCGAAAAGACCCACTCGTTTTTGTCTTCCGTTTTTACGGCGTTTTTTCCTTATTCCCGCTGTGCCTTTACAACCTTATGAGTATGAGAAGGAAA
 Amp Res

5' TTCAATATTATGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATT
 6600
 3' AAGTTATAATAACTTCGTAATAGTCCCAATAACAGAGTACTCGCCTATGTATAAACTTACATAAATCTTTTTATTTGTTTATCCCAAGGCGCGTGTAA
 Amp Res

5' TCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCTTTC
 6700
 3' AGGGGCTTTTTCACGGTGGACTGCAGATTCTTTGGTAATAATAGTACTGTAATTTGATAATTTTATCCGCATAGTGCTCCGGGAAAGCAGAGCGCGCAAG
 Amp Res

5' GGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGT
 6800
 3' CCACACTGCCACTTTTGGAGACTGTGTACGTGAGGGCTCTGCCAGTGTGAACAGACATTTCGCCTACGGCCCTCGTCTGTTCCGGCAGTCCCGCGCA
 Amp Res

5' CAGCGGGTGTGGCGGGTGTGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGACCATATGCGGTGTGAAATACCGCACAGATG
 6900
 3' GTCGCCCAACCGCCACAGCCCCGACCGAATTGATACGCCGTAGTCTCGTCTAACATGACTCTCACGTGGTATACGCCACACTTTATGGCGTGTCTAC
 Amp Res

5' CGTAAGGAGAAAATACCGCATCAGGCGCCATTCCGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTG
 7000
 3' GCATTCTCTTTTATGGCGTAGTCCGGGTAAGCGGTAAGTCCGACGCGTTGACAACCCTTCCCGCTAGCCACGCCCGGAGAAGCGATAATGCGGTGCGAC
 Amp Res

5' GCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAACGACGGCGCAAGGAATGGTGCATGCAA
 7100
 3' CGCTTCCCCCTACACGACGTTCCGCTAATTCAACCCATTGCGGTCCCAAAGGGTCAGTGTGCAACATTTTGTGCGCGGTTCTTACCACGTACGTT
 Amp Res

5' GGAGATGGCGCCCAACAGTCCCCGGCCACGGGGCCTGCCACCATACCCACGCCGAAACAAGCGCTCATGAGCCGAAGTGGCGAGCCCGATCTTCCCA
 7200
 3' CCTCTACCGGGGTTGTCAGGGGGCCGGTGCCCCGGACGGTGGTATGGGTGCGGCTTTGTTCCGAGTACTCGGGCTTACCCTCGGGCTAGAAGGGGT
 Amp Res

