

Supplemental Figure Legends

Supplemental Figure 1. Installation of a reverse-oriented T7 promoter to make *Rev Luc-UTR pcDNA 3.1*

(-). Vector and hybridized oligos containing a T7 promoter and M13 sequencing primer were cut with BamHI and HindIII then ligated together and transformed into *E. coli* with standard molecular biology techniques. Figures are drawn to scale. A black vertical line indicates a restriction site; a gray box represents a T7 promoter; red box, beta-globin untranslated region; green box with arrowhead, optimized luciferase coding region; black horizontal line, plasmid backbone (remaining plasmid not shown). The white slash through the luciferase coding region indicates a break in the sequence to allow the relevant parts of the plasmid drawn to scale.

Supplemental Figure 2. Installation of an internal poly(A) tract to make *Luc-UTR 80A pc DNA3.1* (-).

Vector and two sets of hybridized oligos were cut at the labeled restriction sites, ligated together, and transformed into *E. coli* with standard molecular biology techniques. An A₆₂ tract was expected from this ligation. One clone was identified with an A₃₃ tract installation and the correct surrounding sequence, and this plasmid was used further. Utilizing NEB's Q5 Site Directed Mutagenesis kit and the oligos listed, a BsmBI restriction site was edited out to allow for further construction of the poly(A) tract. The poly(A) tract was then expanded from A₃₃ to A₅₇ to A₈₁ using standard molecular biology techniques. Linearization with BsmBI and in vitro transcription allows for synthesis of an mRNA that contains only adenosine nucleotides in its poly(A) tail.

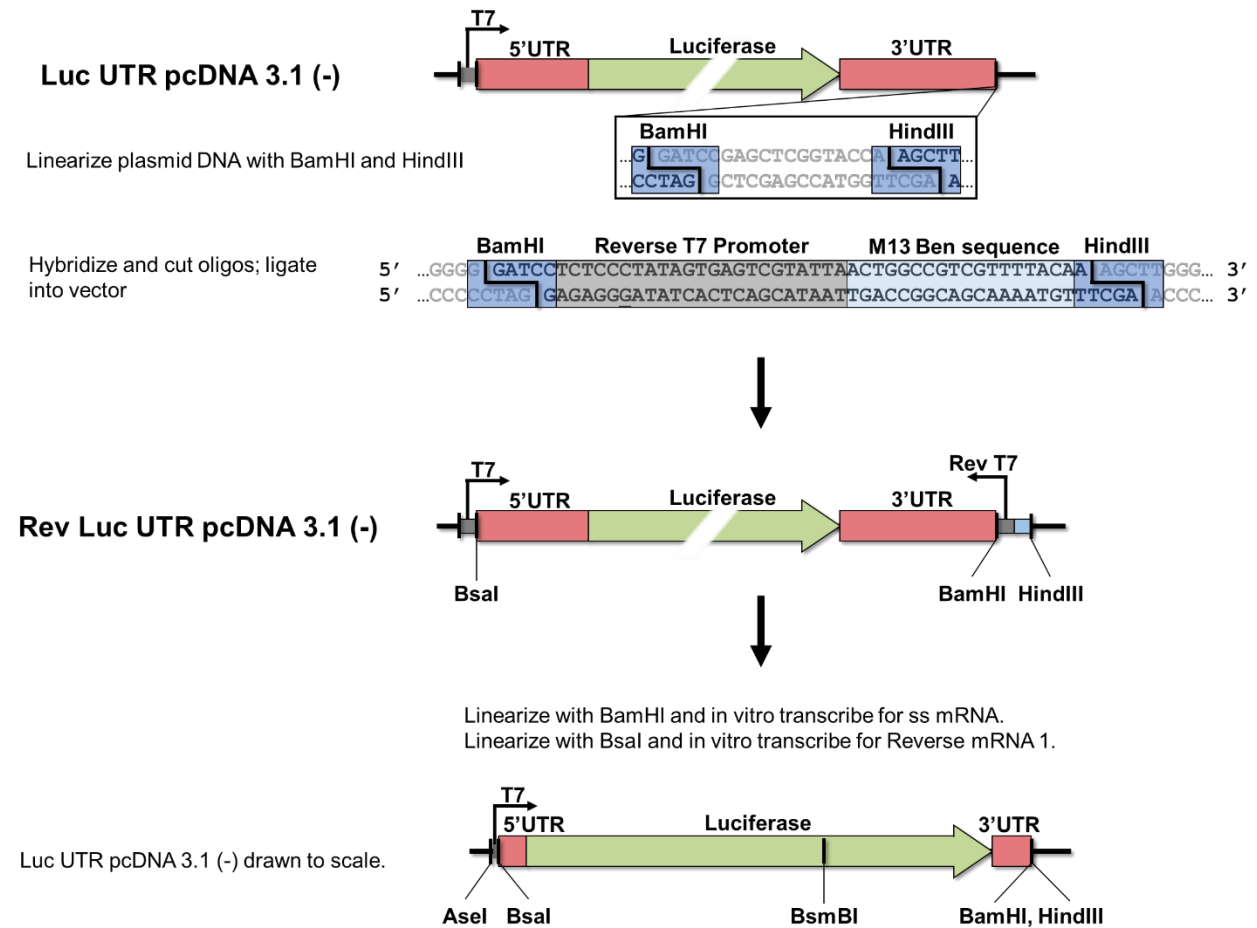
Supplemental Figure 3. Installation of a reverse-oriented T7 promoter to make *Rev Luc-UTR-pcDNA 3.1*

(-). Annealed oligos were ligated into cut vector and transformed into *E. coli* using standard molecular biology techniques.

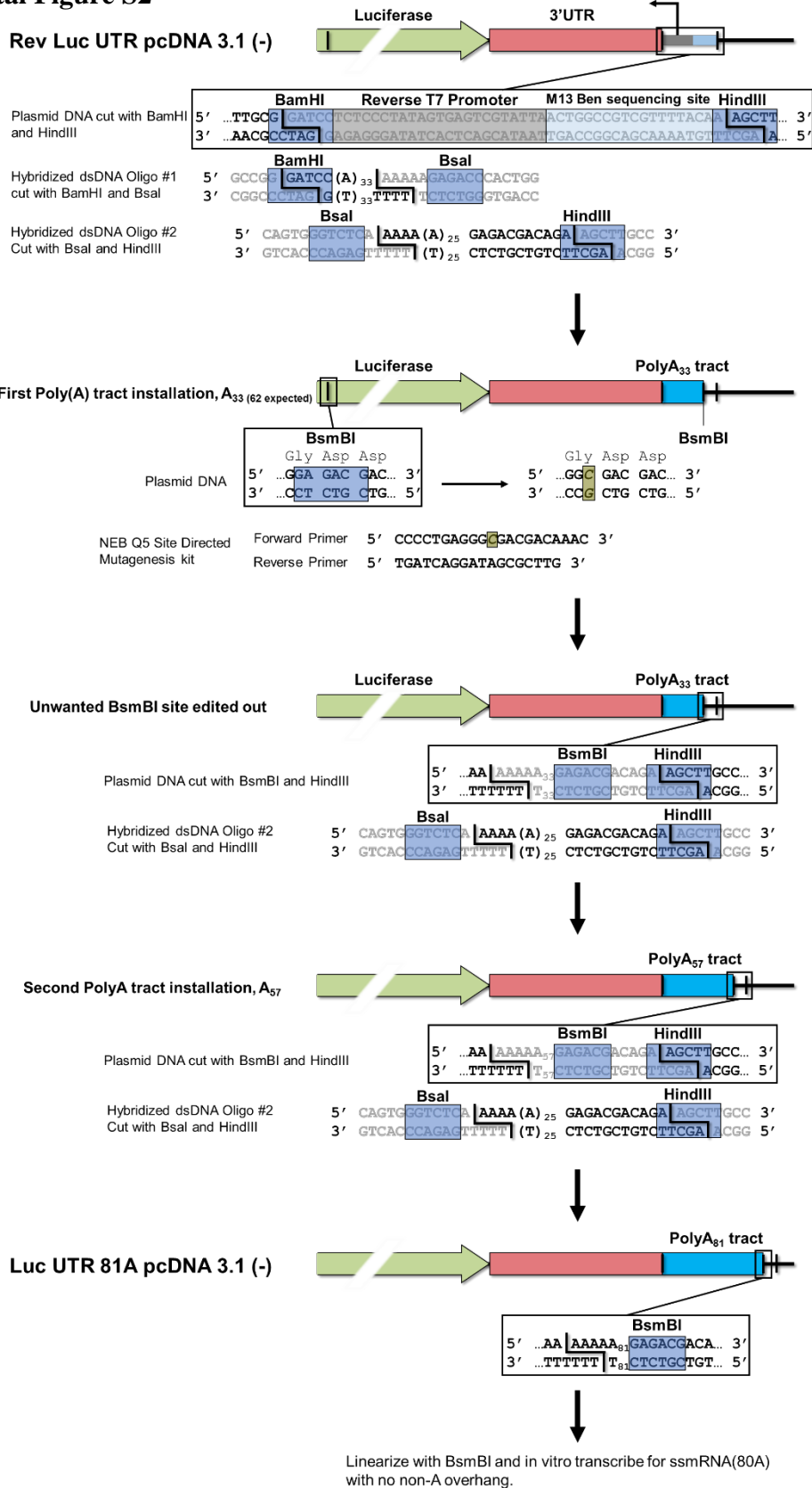
Supplemental Figure 4. PCR-mediated synthesis of *Luc-DNA*. Primers as shown were utilized to amplify the luciferase coding region of *Luc-UTR pcDNA 3.1 (-)* and install the reverse oriented T7 promoter.

Supplemental Figure 5. Full sequence of all mRNAs used in this study. The sequences were aligned using a line splitting program designed by an author (STC). The m7G designates a 7-methylguanylate cap. The mRNA sequences are highlighted according to the key to demarcate the regions of interest.

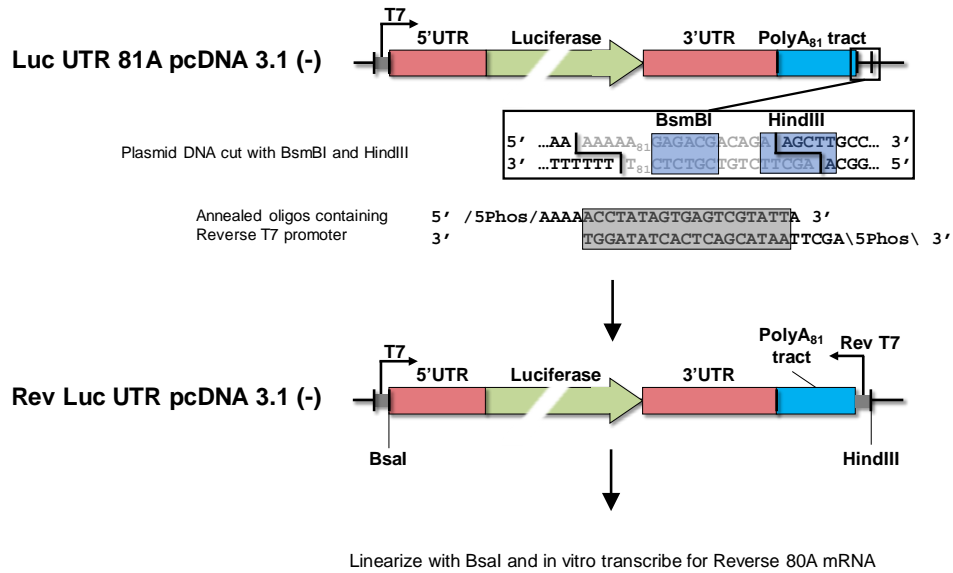
Supplemental Figure S1



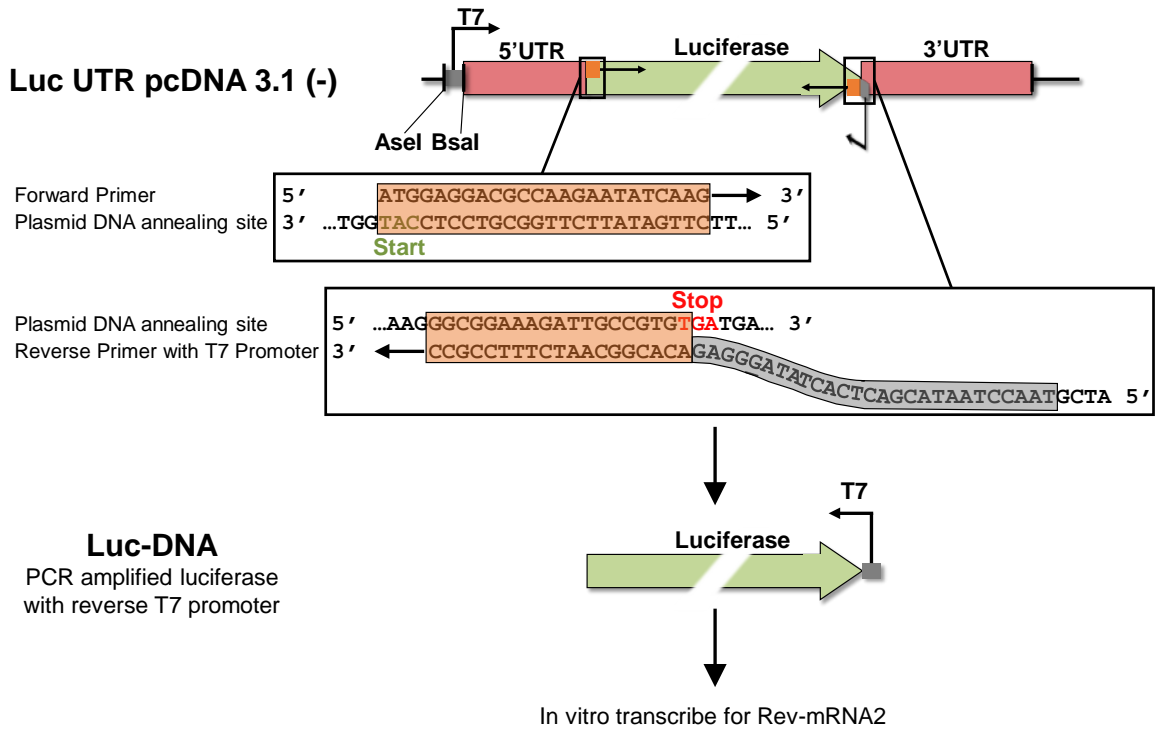
Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4



Supplemental Figure S5

Key: Overhangs on reverse strands Human Beta Globin 5' and 3' UTRs Start Codon Mutated Base Stop Codon

ss mRNA : 5' m7G-GGGAGACCCAAGCUGGCUAGCGUUUAAACGGGCCCCUCUAGAACAUUUGCUUCUGACACA
ss 80A mRNA : 5' m7G-GGGAGACCCAAGCUGGCUAGCGUUUAAACGGGCCCCUCUAGAACAUUUGCUUCUGACACA
Reverse 1 : 3' UAUCCCUCUGGGUUCGACCGAUCGCAAUUUGCCCGGGAGAUCUUUGUAAACGAAGACUGUGU
Reverse 2 : 3'
Reverse 80A : 3' UAUCCCUCUGGGUUCGACCGAUCGCAAUUUGCCCGGGAGAUCUUUGUAAACGAAGACUGUGU

ss mRNA : ACUGUGUUCACUAGCAACCCUCAAACAGACACCGCCGCCACCAUGGAGGACGCCAAGAAUAUCAAGAA
ss 80A mRNA : ACUGUGUUCACUAGCAACCCUCAAACAGACACCGCCGCCACCAUGGAGGACGCCAAGAAUAUCAAGAA
Reverse 1 : UGACACAAGUGAUUCGUUGGAGUUUGUCUGUGGGCGGCGGUGGUACCUCCUGCGGUUCUUAUAGUUCUU
Reverse 2 : UACCUCCUGCGGUUCUUAUAGUUCUU
Reverse 80A : UGACACAAGUGAUUCGUUGGAGUUUGUCUGUGGGCGGCGGUGGUACCUCCUGCGGUUCUUAUAGUUCUU

ss mRNA : AGGCCCCGCUCUCCUUCUACCCCCUGGAAGACGGCACAGCCGGCGAACAACUCCACAAGGCCAUGAAG
ss 80A mRNA : AGGCCCCGCUCUCCUUCUACCCCCUGGAAGACGGCACAGCCGGCGAACAACUCCACAAGGCCAUGAAG
Reverse 1 : UCCGGGGCGAGGGAAGAUGGGGGACCUUCUGCCGUGUCGGCCGCUUGUUGAGGUGUUCGGUACUUC
Reverse 2 : UCCGGGGCGAGGGAAGAUGGGGGACCUUCUGCCGUGUCGGCCGCUUGUUGAGGUGUUCGGUACUUC
Reverse 80A : UCCGGGGCGAGGGAAGAUGGGGGACCUUCUGCCGUGUCGGCCGCUUGUUGAGGUGUUCGGUACUUC

ss mRNA : AGGUAUGCCCUGGUCCCCGGAACCAUCGCCUUCACCGAUGCCCAUAUCGAGGUCGACAUCACCUACG
ss 80A mRNA : AGGUAUGCCCUGGUCCCCGGAACCAUCGCCUUCACCGAUGCCCAUAUCGAGGUCGACAUCACCUACG
Reverse 1 : UCCAUACGGGACCAGGGGCCUUGGUAGCGGAAGUGGCUACGGGUAUAGCUCACGUCUGUAGUGGAUGC
Reverse 2 : UCCAUACGGGACCAGGGGCCUUGGUAGCGGAAGUGGCUACGGGUAUAGCUCACGUCUGUAGUGGAUGC
Reverse 80A : UCCAUACGGGACCAGGGGCCUUGGUAGCGGAAGUGGCUACGGGUAUAGCUCACGUCUGUAGUGGAUGC

ss mRNA : CUGAGUACUUCGAGAUGUCCGUCAGGCUCGCCGAGGCUAUGAAGAGAUUAGGCCUCAACACCAACCA
ss 80A mRNA : CUGAGUACUUCGAGAUGUCCGUCAGGCUCGCCGAGGCUAUGAAGAGAUUAGGCCUCAACACCAACCA
Reverse 1 : GACUCAUGAAGCUCUACAGGCAGUCCGAGCGGCUCCGAUACUUCUCUAUACCGGAGUUGUGGUUGGU
Reverse 2 : GACUCAUGAAGCUCUACAGGCAGUCCGAGCGGCUCCGAUACUUCUCUAUACCGGAGUUGUGGUUGGU
Reverse 80A : GACUCAUGAAGCUCUACAGGCAGUCCGAGCGGCUCCGAUACUUCUCUAUACCGGAGUUGUGGUUGGU

ss mRNA : CAGGAUCGUGGUCUGCUCUCCGAGAACUCCUUGCAGUUUUUCAUGCCCUGUCUGGGCGCCCUGUUUAUC
ss 80A mRNA : CAGGAUCGUGGUCUGCUCUCCGAGAACUCCUUGCAGUUUUUCAUGCCCUGUCUGGGCGCCCUGUUUAUC
Reverse 1 : GUCCUAGCACCAGACGAGGCUCUUGAGGGACGUCAAAAAGUACGGGCACGACCCGCGGGACAAUAG
Reverse 2 : GUCCUAGCACCAGACGAGGCUCUUGAGGGACGUCAAAAAGUACGGGCACGACCCGCGGGACAAUAG
Reverse 80A : GUCCUAGCACCAGACGAGGCUCUUGAGGGACGUCAAAAAGUACGGGCACGACCCGCGGGACAAUAG

ss mRNA : GGAGUCGCCGUCGCCCCCGCUAACGACAUCUACAACGAGAGAGAGCUCUGAACAGCAUGGGCAUUU
ss 80A mRNA : GGAGUCGCCGUCGCCCCCGCUAACGACAUCUACAACGAGAGAGAGCUCUGAACAGCAUGGGCAUUU
Reverse 1 : CCUCAGCGGCAGCGGGGGCGAUUGCUGUAGAUGUUGCUCUCUCUCGAGGACUUGUCGUACCCGUAAA
Reverse 2 : CCUCAGCGGCAGCGGGGGCGAUUGCUGUAGAUGUUGCUCUCUCUCGAGGACUUGUCGUACCCGUAAA
Reverse 80A : CCUCAGCGGCAGCGGGGGCGAUUGCUGUAGAUGUUGCUCUCUCUCGAGGACUUGUCGUACCCGUAAA

ss mRNA : CCCAGCCUACCGUGGUCUUCGUGAGCAAGAAAGGCCUCCAGAAGAUCUGAACGUCUCCAAAAGAAGCU
ss 80A mRNA : CCCAGCCUACCGUGGUCUUCGUGAGCAAGAAAGGCCUCCAGAAGAUCUGAACGUCUCCAAAAGAAGCU
Reverse 1 : GGGUCGGAUGGCACCAGAAGCACUCGUUCUUCUCCGGAGGUCUUCUAGGACUUGCAGGUUUUCUUCGA
Reverse 2 : GGGUCGGAUGGCACCAGAAGCACUCGUUCUUCUCCGGAGGUCUUCUAGGACUUGCAGGUUUUCUUCGA
Reverse 80A : GGGUCGGAUGGCACCAGAAGCACUCGUUCUUCUCCGGAGGUCUUCUAGGACUUGCAGGUUUUCUUCGA

ss mRNA : GCCCAUUAUCCAGAAGAUCAUCAUCAUGGAUAGCAAGACCGACUACCAGGGAUUCAGUCCAUGUAC

ss 80A mRNA : GCCCAUUAUCCAGAAGAUAUCAUCAUGGAUAGCAAGACCGACUACCAGGGAUUCAGUCCAUGUAC
 Reverse 1 : CGGGUAAUAGGUCUUCUAGUAGUAGUACCUAUCGUUCUGGCUGAUGGUCCUAAGGUCAGGUACAUG
 Reverse 2 : CGGGUAAUAGGUCUUCUAGUAGUAGUACCUAUCGUUCUGGCUGAUGGUCCUAAGGUCAGGUACAUG
 Reverse 80A : CGGGUAAUAGGUCUUCUAGUAGUAGUACCUAUCGUUCUGGCUGAUGGUCCUAAGGUCAGGUACAUG

ss mRNA : ACCUUCGUGACAAGCCAUCUGCCCCCGGCUUCAACGAGUAUGACUUCGUCCCCGAGUCCUUCGACA
 ss 80A mRNA : ACCUUCGUGACAAGCCAUCUGCCCCCGGCUUCAACGAGUAUGACUUCGUCCCCGAGUCCUUCGACA
 Reverse 1 : UGGAAGCACUGUUCGGUAGACGGGGGGCCGAAGUUGCUCUAUCUGAAGCAGGGGCUCAGGAAGCUGU
 Reverse 2 : UGGAAGCACUGUUCGGUAGACGGGGGGCCGAAGUUGCUCUAUCUGAAGCAGGGGCUCAGGAAGCUGU
 Reverse 80A : UGGAAGCACUGUUCGGUAGACGGGGGGCCGAAGUUGCUCUAUCUGAAGCAGGGGCUCAGGAAGCUGU

ss mRNA : GAGACAAGACCAUCGCCUGAUCAUGAACUCCUCCGGAAGCACCGGACUGCCCAAAGGCGUGGCUCU
 ss 80A mRNA : GAGACAAGACCAUCGCCUGAUCAUGAACUCCUCCGGAAGCACCGGACUGCCCAAAGGCGUGGCUCU
 Reverse 1 : CUCUGUUCUGGUAGCGGGACUAGUACUUGAGGAGGCCUUCGUGGCCUGACGGGUUUCGACCCGAGA
 Reverse 2 : CUCUGUUCUGGUAGCGGGACUAGUACUUGAGGAGGCCUUCGUGGCCUGACGGGUUUCGACCCGAGA
 Reverse 80A : CUCUGUUCUGGUAGCGGGACUAGUACUUGAGGAGGCCUUCGUGGCCUGACGGGUUUCGACCCGAGA

ss mRNA : CCCUCACAGGACCGCUUGUGUCAGGUUCAGCCACGCCAGGGACCCCAUCUUCGGCAACCAGAUCAUC
 ss 80A mRNA : CCCUCACAGGACCGCUUGUGUCAGGUUCAGCCACGCCAGGGACCCCAUCUUCGGCAACCAGAUCAUC
 Reverse 1 : GGGAGUGUCCUGGCGAACACAGUCCAAGUCGGUGCGGUCCUGGGGUAGAAGCCGUUGGUCUAGUAG
 Reverse 2 : GGGAGUGUCCUGGCGAACACAGUCCAAGUCGGUGCGGUCCUGGGGUAGAAGCCGUUGGUCUAGUAG
 Reverse 80A : GGGAGUGUCCUGGCGAACACAGUCCAAGUCGGUGCGGUCCUGGGGUAGAAGCCGUUGGUCUAGUAG

ss mRNA : CCCGACACAGCUAUCCUCAGCGUGGUGCCCUUCCACCACGGCUUCGGAAUGUUCACCACCCUCGGCU
 ss 80A mRNA : CCCGACACAGCUAUCCUCAGCGUGGUGCCCUUCCACCACGGCUUCGGAAUGUUCACCACCCUCGGCU
 Reverse 1 : GGGCUGUGUCGAUAGGAGUCGCACCACGGGAAGGUGGUGCCGAAGCCUUAACAAGUGGUGGGAGCCGA
 Reverse 2 : GGGCUGUGUCGAUAGGAGUCGCACCACGGGAAGGUGGUGCCGAAGCCUUAACAAGUGGUGGGAGCCGA
 Reverse 80A : GGGCUGUGUCGAUAGGAGUCGCACCACGGGAAGGUGGUGCCGAAGCCUUAACAAGUGGUGGGAGCCGA

ss mRNA : ACCUCAUCUGUGGCUUCAGAGUGGUGCUCAUGUACAGAUUCGAGGAGGAGCUGUUUCUGAGGUCCCU
 ss 80A mRNA : ACCUCAUCUGUGGCUUCAGAGUGGUGCUCAUGUACAGAUUCGAGGAGGAGCUGUUUCUGAGGUCCCU
 Reverse 1 : UGGAGUAGACACCGAAGUCUACACCACGAGUACAUGUCUAAGCUCUCCUCGACAAAGACUCCAGGGA
 Reverse 2 : UGGAGUAGACACCGAAGUCUACACCACGAGUACAUGUCUAAGCUCUCCUCGACAAAGACUCCAGGGA
 Reverse 80A : UGGAGUAGACACCGAAGUCUACACCACGAGUACAUGUCUAAGCUCUCCUCGACAAAGACUCCAGGGA

ss mRNA : CCAGGACUACAAAUCCAAUCCGCUCUGCUCGUCCCCACCCUGUUCAGCUUCUUCGCCAAAAGCACC
 ss 80A mRNA : CCAGGACUACAAAUCCAAUCCGCUCUGCUCGUCCCCACCCUGUUCAGCUUCUUCGCCAAAAGCACC
 Reverse 1 : GGUCCUGAUGUUUAGGUUAGGCGAGACGAGCAGGGGUGGGACAAGUCGAAGAAGCGGUUUUCGUGG
 Reverse 2 : GGUCCUGAUGUUUAGGUUAGGCGAGACGAGCAGGGGUGGGACAAGUCGAAGAAGCGGUUUUCGUGG
 Reverse 80A : GGUCCUGAUGUUUAGGUUAGGCGAGACGAGCAGGGGUGGGACAAGUCGAAGAAGCGGUUUUCGUGG

ss mRNA : CUGAUCGACAAGUAUGACCUCUCCAACCUGCAUGAGAUCGCCAGCGGAGGAGCCCCUCUGUCCAAGG
 ss 80A mRNA : CUGAUCGACAAGUAUGACCUCUCCAACCUGCAUGAGAUCGCCAGCGGAGGAGCCCCUCUGUCCAAGG
 Reverse 1 : GACUAGCUGUUCAUACUGGAGAGGUUGGACGUACUCUAGCGGUCGCCUCCUCGGGGAGACAGGUUCC
 Reverse 2 : GACUAGCUGUUCAUACUGGAGAGGUUGGACGUACUCUAGCGGUCGCCUCCUCGGGGAGACAGGUUCC
 Reverse 80A : GACUAGCUGUUCAUACUGGAGAGGUUGGACGUACUCUAGCGGUCGCCUCCUCGGGGAGACAGGUUCC

ss mRNA : AGGUCGGCGAAGCCGUGGCUAAGAGGUUUCACCUCCUGGCAUUAAGGCAAGGAUACGGCCUGACCGA
 ss 80A mRNA : AGGUCGGCGAAGCCGUGGCUAAGAGGUUUCACCUCCUGGCAUUAAGGCAAGGAUACGGCCUGACCGA
 Reverse 1 : UCCAGCCGCUUCGGCACCGAUUCUCCAAGUGGAGGGACCGUAAUCCGUUCCUAUGCCGGACUGGCU
 Reverse 2 : UCCAGCCGCUUCGGCACCGAUUCUCCAAGUGGAGGGACCGUAAUCCGUUCCUAUGCCGGACUGGCU
 Reverse 80A : UCCAGCCGCUUCGGCACCGAUUCUCCAAGUGGAGGGACCGUAAUCCGUUCCUAUGCCGGACUGGCU

ss mRNA : AACCACAAGCGCUAUCCUGAUCACCCUGAGGGAGACGACAAACCCGGAGCCGUCGGAAAGGUCGUC
 ss 80A mRNA : AACCACAAGCGCUAUCCUGAUCACCCUGAGGGGACGACAAACCCGGAGCCGUCGGAAAGGUCGUC
 Reverse 1 : UUGGUGUUCGCGAUAGGACUAGUGGGGACUCCUCUGCUGUUGGGCCUCGGCAGCCUUUCAGCAG
 Reverse 2 : UUGGUGUUCGCGAUAGGACUAGUGGGGACUCCUCUGCUGUUGGGCCUCGGCAGCCUUUCAGCAG
 Reverse 80A : UUGGUGUUCGCGAUAGGACUAGUGGGGACUCCUCUGCUGUUGGGCCUCGGCAGCCUUUCAGCAG

ss mRNA : CCCUUCUUCGAGGCCAAGGUGGUCGACCCUGGACACCGCAAGACCCUGGGCGUGAACC AAAGGGGCG
 ss 80A mRNA : CCCUUCUUCGAGGCCAAGGUGGUCGACCCUGGACACCGCAAGACCCUGGGCGUGAACC AAAGGGGCG
 Reverse 1 : GGAAGAAGCUC CGGUUCCACCAGCUGGACCUUGGGCCGUUCUGGGACCCGCACUUGGUUUC CCGC
 Reverse 2 : GGAAGAAGCUC CGGUUCCACCAGCUGGACCUUGGGCCGUUCUGGGACCCGCACUUGGUUUC CCGC
 Reverse 80A : GGAAGAAGCUC CGGUUCCACCAGCUGGACCUUGGGCCGUUCUGGGACCCGCACUUGGUUUC CCGC

ss mRNA : AACUCUGUGAGGGGCCCUAUGAUCAUGAGCGGCUACGUCAACAACCCUGAGGCUACCAACGCUCU
 ss 80A mRNA : AACUCUGUGAGGGGCCCUAUGAUCAUGAGCGGCUACGUCAACAACCCUGAGGCUACCAACGCUCU
 Reverse 1 : UUGAGACACACUCCCCGGGAUACUAGUACUCGCCGAUGCAGUUGUUGGGACUCCGAUGGUUGCGAGA
 Reverse 2 : UUGAGACACACUCCCCGGGAUACUAGUACUCGCCGAUGCAGUUGUUGGGACUCCGAUGGUUGCGAGA
 Reverse 80A : UUGAGACACACUCCCCGGGAUACUAGUACUCGCCGAUGCAGUUGUUGGGACUCCGAUGGUUGCGAGA

ss mRNA : CAUCGACAAGGAUGGCUGGCUGCACUCCGGAGAUAUUCGCCUACUGGGACGAGGAUGAGCAUUUUUU
 ss 80A mRNA : CAUCGACAAGGAUGGCUGGCUGCACUCCGGAGAUAUUCGCCUACUGGGACGAGGAUGAGCAUUUUUU
 Reverse 1 : GUAGCUGUUCUACCGACCCGACGUGAGGCCUCUAUAGCGGAUGACCCUGCUCUACUCGUAAAAAAA
 Reverse 2 : GUAGCUGUUCUACCGACCCGACGUGAGGCCUCUAUAGCGGAUGACCCUGCUCUACUCGUAAAAAAA
 Reverse 80A : GUAGCUGUUCUACCGACCCGACGUGAGGCCUCUAUAGCGGAUGACCCUGCUCUACUCGUAAAAAAA

ss mRNA : AUCGUCGAUAGGCUCAAGAGCCUGAUC AAGUACAAGGGCUACCAGGUGGCCCCUGCUGAGCUCGAAU
 ss 80A mRNA : AUCGUCGAUAGGCUCAAGAGCCUGAUC AAGUACAAGGGCUACCAGGUGGCCCCUGCUGAGCUCGAAU
 Reverse 1 : UAGCAGCUAUCCGAGUUCUCGGACUAGUUCAUGUUC CCGAUGGUCCACCGGGGACGACUCGAGCUUA
 Reverse 2 : UAGCAGCUAUCCGAGUUCUCGGACUAGUUCAUGUUC CCGAUGGUCCACCGGGGACGACUCGAGCUUA
 Reverse 80A : UAGCAGCUAUCCGAGUUCUCGGACUAGUUCAUGUUC CCGAUGGUCCACCGGGGACGACUCGAGCUUA

ss mRNA : CCAUCCUGCUC CAGCACCCUAACAUCUUGACGCGUGGCGUGGCGUGGACUGCCUGACGACGACGCGUGG
 ss 80A mRNA : CCAUCCUGCUC CAGCACCCUAACAUCUUGACGCGUGGCGUGGCGUGGACUGCCUGACGACGACGCGUGG
 Reverse 1 : GGUAGGACGAGGUCGUGGGAUUGUAGAAACUGCGACCCGACCCGACCCUGACGACGACGACGCGACC
 Reverse 2 : GGUAGGACGAGGUCGUGGGAUUGUAGAAACUGCGACCCGACCCGACCCUGACGACGACGACGCGACC
 Reverse 80A : GGUAGGACGAGGUCGUGGGAUUGUAGAAACUGCGACCCGACCCGACCCUGACGACGACGACGCGACC

ss mRNA : CGAACUCCUGCCGUCUGCUGGUCUCGAACACGGCAAGACA AUGACCGAGAAGGAGAUUCGUGGAC
 ss 80A mRNA : CGAACUCCUGCCGUCUGCUGGUCUCGAACACGGCAAGACA AUGACCGAGAAGGAGAUUCGUGGAC
 Reverse 1 : GCUUGAGGGACGGCGACAGCACCAGGAGCUUGUGCCGUUCUGUACUGGCUCUUCUCUAGCACCUG
 Reverse 2 : GCUUGAGGGACGGCGACAGCACCAGGAGCUUGUGCCGUUCUGUACUGGCUCUUCUCUAGCACCUG
 Reverse 80A : GCUUGAGGGACGGCGACAGCACCAGGAGCUUGUGCCGUUCUGUACUGGCUCUUCUCUAGCACCUG

ss mRNA : UACGUGGCCUCCCAAGUGACAACAGCCAAGAAGCUGAGAGGGCGGAGUGGUGUUCGUGGACGAGGUGC
 ss 80A mRNA : UACGUGGCCUCCCAAGUGACAACAGCCAAGAAGCUGAGAGGGCGGAGUGGUGUUCGUGGACGAGGUGC
 Reverse 1 : AUGCACC GGAGGGUUCACUGUUGUCGGUUCUUCGACUCUCCGCCUCACCACAAGCACCUGCUC CACG
 Reverse 2 : AUGCACC GGAGGGUUCACUGUUGUCGGUUCUUCGACUCUCCGCCUCACCACAAGCACCUGCUC CACG
 Reverse 80A : AUGCACC GGAGGGUUCACUGUUGUCGGUUCUUCGACUCUCCGCCUCACCACAAGCACCUGCUC CACG

ss mRNA : CCAAGGGCCUGACAGGCAAGCUCGACGCUAGAAAGAU CAGGGAGAUUCUGAUUAAAGCCAAAAGGG
 ss 80A mRNA : CCAAGGGCCUGACAGGCAAGCUCGACGCUAGAAAGAU CAGGGAGAUUCUGAUUAAAGCCAAAAGGG
 Reverse 1 : GGUUCCCGGACUGCCGUUCGAGCUGCGAUUUUCUAGUCCUCUAAGACUAAUUUCGGUUUUUCC

