

>Supplemental File 1: Insulator (dimers), promoter (monomers) and primer sequences

>Sequences used (dimers for insulator activity, monomers for promoter activity): scs' sequences in uppercase, mutant and linker sequences in lowercase, BEAF-32B binding site CGATA motifs underlined.

-----1-----2-----3-----4-----5-----6-----7-----8-----9-----0

>Insulator sequences: except scs', all were tested as the indicated dimers and were BglIII-BamHI fragments cloned into the BamHI site of pC4-scs or pC4-attB-scs.

>scs'

tctagaactagtgatccccCTAGAGAATTCCAACAAAACCTTTGCTTGTTGATAACACCTGATGTTGAGAGATGCCGATAAAATCACAGCTGTTCTG  
GTTTACAGTCACCAGAAATAAAAAATATTGGAATTGAGATGTACACAATTAACGATATTTATAAATATCTTCCGATAGTCTATCGTCCGGTTAATCAAAA  
TAAAGTGCACGAATTAACATATTTTCAAATTAAGACGCTTTGATAGATGATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAG  
TTTAGAGCACTATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCG  
ATAGTATCGCACACTTGCCATTGCGCTCATCACGCACACGCCCGCTTACAATTCAAACGAACGGCATTATTTTACAGGATCCCGGGAGTCGTGAAT  
GTTTTACCAATATCGACTTTCATTGTTCTCTAGgggctcgacctcgag

>M2

agatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCTTGATTTCAAAT  
TGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCGCTCATCACGCAC  
ACGCCCGCTTACAATTCAAACGAACGGatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAGCACT  
ATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCA  
CACTTGCCATTGCGCTCATCACGCACACGCCCGCTTACAATTCAAACGAACGGatcc

>M\*2 (aka H\*2)

agatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCTTGATTTCAAAT  
TGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCactcgATTCTTCCACCAACctcgAGTcgaGCACACTTGCCATTGCGCTCATCACGCAC  
ACGCCCGCTTACAATTCAAACGAACGGatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAGCACT  
ATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCactcgATTCTTCCACCAACctcgAGTcgaGCA  
CACTTGCCATTGCGCTCATCACGCACACGCCCGCTTACAATTCAAACGAACGGatcc

>S2

agatctaagcttCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCGCTCATCACGCACACGCCCGCTTACA  
ATTCAAACGAACGGatctaagcttCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCGCTCATCACGCACAC  
GCCCGCTTACAATTCAAACGAACGGatcc

>X2

ggatcCAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTagatcCAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTagat  
ct

>LS1-2

agatctaagcttaGgtaataacTAGtGcctGcAtTTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCTTGATTTCAAAT

TGAAATAATACACAATATAACATTTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCAC  
ACGCCCCGCTTCACAATTCAAACGAACGGatctaagcttaGgtaataaacTAGtGcctGcAtTTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAGCACT  
ATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCA  
CACTTGCCATTGCCTCATCACGCACACGCCCCGCTTCACAATTCAAACGAACGGatcc

>LS2-2

agatctaagcttTGTATTTGTATAGAGATAGAggTaAtaacTAgtgcctgcatAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCTTGATTTCAAAT  
TGAAATAATACACAATATAACATTTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCAC  
ACGCCCCGCTTCACAATTCAAACGAACGGatctaagcttTGTATTTGTATAGAGATAGAggTaAtaacTAgtgcctgcatAATGCCAAAGTTTAGAGCACT  
ATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCA  
CACTTGCCATTGCCTCATCACGCACACGCCCCGCTTCACAATTCAAACGAACGGatcc

>LS3-2

agatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACAggtAataactAGTgcctgcatCTATTCAATAATTCTCTTGATTTCAAAT  
TGAAATAATACACAATATAACATTTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCAC  
ACGCCCCGCTTCACAATTCAAACGAACGGatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACAggtAataactAGTgcctgcatCT  
ATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCA  
CACTTGCCATTGCCTCATCACGCACACGCCCCGCTTCACAATTCAAACGAACGGatcc

>LS4-2

agatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAaggtaATAactagtgcCTgcatATTTCAAAT  
TGAAATAATACACAATATAACATTTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCAC  
ACGCCCCGCTTCACAATTCAAACGAACGGatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAaggta  
ATAactagtgcCTgcatATTTCAAATTGAAATAATACACAATATAACATTTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCA  
CACTTGCCATTGCCTCATCACGCACACGCCCCGCTTCACAATTCAAACGAACGGatcc

>LS5-2

agatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCagGtaaTaActa  
gtgccTgcatCACAATATAACATTTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCAC  
ACGCCCCGCTTCACAATTCAAACGAACGGatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAGCACT  
ATTCAATAATTCTCagGtaaTaActagtgccTgcatCACAATATAACATTTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCA  
CACTTGCCATTGCCTCATCACGCACACGCCCCGCTTCACAATTCAAACGAACGGatcc

>LS6-2

agatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCTTGATTTCAAAT  
TGAAATAggtaAtAAactagtgccTgcaTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCAC  
ACGCCCCGCTTCACAATTCAAACGAACGGatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTAGAGCACT  
ATTCAATAATTCTCTTGATTTCAAATTGAAATAggtaAtAAactagtgccTgcaTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCA  
CACTTGCCATTGCCTCATCACGCACACGCCCCGCTTCACAATTCAAACGAACGGatcc

>Δ5-2

agatctaagcttTTAGAGCACTATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTTCTAACACTACAAAGTCACGATATTCT

TCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCACACGCCCGCTTCACAATTCAAACGAACGGatctaagcttTTAGAGCACTATTC  
AATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACT  
TGCCATTGCCTCATCACGCACACGCCCGCTTCACAATTCAAACGAACGGatcc

>Δ3-2

agatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTGTAGAGCACTATTCAATAATTCTCTTGATTTCAAAT  
TGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACgcatctaagcttTGTATTTGTAT  
AGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTGTAGAGCACTATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACAT  
TTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACgcatcc

>Δ5Δ3-2

agatctaagcttTTAGAGCACTATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCT  
TCCACCAACCGATAGTATCGCACACgcatctaagcttTTAGAGCACTATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTCT  
TAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACgcatcc

>Sp\*-2

agatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTGTAGAGCACTATTCAATAATTCTCTTGATTTCCaAc  
aGgtAagAcAgttcgcAggtaATaactagtgcCTgCatAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCAC  
ACGCCCGCTTCACAATTCAAACGAACGGatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTGTAGAGCACT  
ATTCAATAATTCTCTTGATTTCCaAcaGgtAagAcAgttcgcAggtaATaactagtgcCTgCatAGTCACGATATTCTTCCACCAACCGATAGTATCGCA  
CACTTGCCATTGCCTCATCACGCACACGCCCGCTTCACAATTCAAACGAACGGatcc

>ΔSp-2

agatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTGTAGAGCACTATTCAATAATTCTCTTGATTTCCAGTC  
ACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCACACGCCCGCTTCACAATTCAAACGAACGGatctaagcttTGTA  
TTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTGTAGAGCACTATTCAATAATTCTCTTGATTTCCAGTCACGATATTCTTCCACC  
AACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCACACGCCCGCTTCACAATTCAAACGAACGGatcc

-----1-----2-----3-----4-----5-----6-----7-----8-----9-----0

>Luciferase promoter test sequences, including 5' pBSKS sequences and 3' sequences to luciferase ATG

>All were cloned into a SalI site using Gibson assembly.

>M (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaATG)  
gccccccctcgaggTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTGTAGAGCACTATTCAATAATTCTCTTGATTTCAA  
ATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGC  
ACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataagcttcaaaATG

>M\* (aka H\*) (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaATG)  
gccccccctcgaggTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAATGCCAAAGTTTGTAGAGCACTATTCAATAATTCTCTTGATTTCAA  
ATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCAatcgATTCTTCCACCAACcctcgAGTcgaGCACACTTGCCATTGCCTCATCACGC  
ACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataagcttcaaaATG

>LS4\* H\* (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaaATG)  
gccccccctcgaggTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAAATGCCAAAGTTTAGAaggtaATAactagtgcTgcatATTTCAA  
ATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGC  
ACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataagcttcaaaaATG

>LS1 (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaaATG)  
gccccccctcgaggGgtaataacTAGtGcctGcAtTTAAGGTTAAAATAACATAAAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCTTGATTTCAA  
ATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGC  
ACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataagcttcaaaaATG

>LS2 (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaaATG)  
gccccccctcgaggTGTATTTGTATAGAGATAGAggTaAtaacTAgtgcctgcatAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCTTGATTTCAA  
ATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGC  
ACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataagcttcaaaaATG

>LS3 (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaaATG)  
gccccccctcgaggTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACAggtAataactAGTgcctgcatCTATTCAATAATTCTCTTGATTTCAA  
ATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGC  
ACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataagcttcaaaaATG

>LS4 (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaaATG)  
gccccccctcgaggTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAAATGCCAAAGTTTAGAaggtaATAactagtgcTgcatATTTCAA  
ATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGC  
ACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataagcttcaaaaATG

>LS5 (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaaATG)  
gccccccctcgaggTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCagGtaaTaAc  
tagtgccTgcatCACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGC  
ACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataagcttcaaaaATG

>LS6 (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaaATG)  
gccccccctcgaggTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCTTGATTTCAA  
ATTGAAATAggtaAtAAactagtgccTgcaTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGC  
ACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataagcttcaaaaATG

>Δ5 (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaaATG)  
gccccccctcgaggTTAGAGCACTATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATT  
CTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataagcttcaaaaATG

>Δ3 (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaaATG)  
gccccccctcgaggTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCTTGATTTCAA

ATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACcggtatcgataagcttcaaaATG

>Δ5Δ3 (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaATG)  
gccccccctcgaggTTAGAGCACTATTCAATAATTCTCTTGATTTCAAATTGAAATAATACACAATATAACATTTTCTAACACTACAAAGTCACGATATT  
CTTCCACCAACCGATAGTATCGCACACcggtatcgataagcttcaaaATG

>Δ5Δ3 Sp\* (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaATG)  
gccccccctcgaggTTAGAGCACTATTCAATAATTCTCTTGATTTCCaacaggtaagacagttcgcaggtaataACTAGTgcctgcatAGTCACGATATT  
CTTCCACCAACCGATAGTATCGCACACcggtatcgataagcttcaaaATG

>Δ5Δ3ΔSp (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaATG)  
gccccccctcgaggTTAGAGCACTATTCAATAATTCTCTTGATTTCCAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACcggtatcgataagct  
tcaaaATG

>Sp\* (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaATG)  
gccccccctcgaggTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCTTGATTTCCa  
AcaGgtAagAcAgttcgcAggtaATAactagtgcCTgCaTAgTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGC  
ACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataagcttcaaaATG

>ΔSp (5' pBS homology: gccccccctcgagg ; 3' homology to luciferase ATG: cggtatcgataagcttcaaaATG)  
gccccccctcgaggTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAAATGCCAAAGTTTAGAGCACTATTCAATAATTCTCTTGATTTCCAG  
TCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTGCCTCATCACGCACACGCCCGCTTCACAATTCAAACGAACGGcggtatcgataa  
gcttcaaaATG

Linker-scanning mutagenesis of the scs' M fragment:

22 bp substitution with an SpeI site in the middle, adjacent mutations overlap by 3 bp.

Lambda phage 44739-44760: aggtaatagtttagagcctgcat with SpeI: aggtaataACTAGTgcctgcat

Spacer mutation: Lambda 44789-44800; 44731-44746; SpeI; 44753-44760

Spacer mutation: caacaggtaagacagttcgcaggtaataACTAGTgcctgcat

Lambda: 44725 ccgaaacagttcgcaggtaatagtttagagcctgcataacgggtttcgggattttttatatactgcaacaacaggtaagagcat

Lambda: 44788 acaacaggtaagagcat

Primer Sequence (from 5' end)

[Restriction site cloning](#)

M-5 GCA GAT CTA AGC TTT GTA TTT GTA TAG AGA TAG

MS-3 CGG ATC CGT TCG TTT GAA TTG TGA AG

S-5 GCA GAT CTA AGC TTC TAA CAC TAC AAA GTC AC

X-5 gat cCA AAG TCA CGA TAT TCT TCC ACC AAC CGA TAG TAT CGC ACA Cta gat c

X-3 gat ctA GTG TGC GAT ACT ATC GGT TGG TGG AAG AAT ATC GTG ACT TTG gat c

LS1-5 taa cTA GtG cct GcA tTT AAG GTT AAA ATA ACA TAA

LS1-3 gca cta gtt att acc tAA GCT TAG ATC Tga att cgt

LS2-5 taa cTA gtg cct gca tAA TGC CAA AGT TTA GAG CAC

LS2-3 gca cta gtt att acc tCT ATC TCT ATA CAA ATA CAa

LS3-5 taa ctA GTg cct gca tCT ATT CAA TAA TTC TCT TGA

LS3-3 gca cta gtt att acc tGT TAT TTT AAC CTT AAT TTC

LS4-5 Taa cta gtg cCT gca tAT TTC AAA TTG AAA TAA TAC ACA

LS4-3 gca cta gtt att acc tTC TAA ACT TTG GCA TTT ATG TTA

LS5-5 TaA cta gtg ccT gca tCA CAA TAT AAC ATT TTC TAA

LS5-3 gca cta gtt att acc tGA GAA TTA TTG AAT AGT GCT

LS6-5 tAA cta gtg ccT gca TAA CAC TAC AAA GTC ACG ATA

LS6-3 gca cta gtt att acc tAT TTC AAT TTG AAA TCA AGA

Scs'-M-5'del-5-Bgl CCA GAT CTA AGC TTT TAG AGC ACT ATT CAA TAA TTC TCT TG

Scs'-M-5'del-3-Bam GTG GAT CCG TTC GTT TGA ATT GTG AAG C

Scs'-M-3'del-5-Bgl CCA GAT CTA AGC TTT GTA TTT GTA TAG AGA TAG AAA TTA AGG

Scs'-M-3'del-3-Bam GTG GAT CCG TGT GCG ATA CTA TCG GTT G

Scs'-LS4-D-5-Bgl CCA GAT CTA AGC TTT TAG AGC ACT ATT CAA TAA TTC TCT TGA TTT CAG TCA CGA TAT TC

Scs'-LS4-D-3-Bam GTG GAT CCG TGT GCG ATA CTA TCG GTT GGT GGA AGA ATA TCG TGA CTG AAA TCA AGA G

Scs'-LS4-spacer-D-5-Bgl CCA GAT CTA AGC TTT TAG AGC ACT ATT CAA TAA TTC TCT TGA TTT CCA ACA GGT AAG  
ACA GTT CGC AGG TAA TAA CTA G

Scs'-LS4-spacer-D-3-Bam GTG GAT CCG TGT GCG ATA CTA TCG GTT GGT GGA AGA ATA TCG TGA CTA TGC AGG CAC  
TAG TTA TTA CCT GCG AAC TGT C

Gene fragments for making M with Spacer deletion or mutation (double-stranded)

M-spacer-Mut gaacctagatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAAATGCCAAAGTTTAGAGCA  
CTATTCAATAATTCTCTTGATTTCCaacaggtaagacagttcgcaggtaataACTAGTgcctgcatAGTCACGATATT  
CTTCCACCAACCGATAGTATCGCACACTTGCCATTCGCTCATCACGCACACGCCCCGCTTACAATTCAAACGAACGG  
atcctcatgg

M-spacer-Del gaacctagatctaagcttTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAAATGCCAAAGTTTAGAGCA  
CTATTCAATAATTCTCTTGATTTCCAGTCACGATATTCTTCCACCAACCGATAGTATCGCACACTTGCCATTCGCTCA  
TCACGCACACGCCCCGCTTACAATTCAAACGAACGGatcctcatgg

Gibson assembly cloning

M-LS1-Luc-5 gccccccctcgagg aGgtaataacTAGtGcctGcAtTTAAGGTTA  
M-LS2-Luc-5 gccccccctcgagg TGTATTTGTATAGAGATAGAggTaAtaacTA  
M-Luc-5 gccccccctcgagg TGTATTTGTATAGAGATAGAAATTAAGGTTA  
M-Luc-del5-5 gccccccctcgagg TTAGAGCACTATTCAATAATTCTCTTG  
M-Luc-3 CATTTTGAAGCTTATCGATACCG CCGTTCGTTTGAATTGTGAAG  
M-Luc-del3-3 CATTTTGAAGCTTATCGATACCG GTGTGCGATACTATCGGTTG  
rev-M-Luc-5 gccccccctcgagg CCGTTCGTTTGAATTGTGAAG  
rev-M-Luc-3 CATTTTGAAGCTTATCGATACCG TGTATTTGTATAGAGATAGAAATTAAGGTTA

[Ultramers for making M with Spacer deletion or mutation from del5del3 with Spacer deletion or mutation](#)

full-M-5 gccccccctcgaggTGTATTTGTATAGAGATAGAAATTAAGGTTAAAATAACATAAAATGCCAAAGTTTAGAGCACTAT  
TCAATAATTCTCTTG  
full-M-3 CATTTTGAAGCTTATCGATACCGCCGTTTCGTTTGAATTGTGAAGCGGGCGTGTGCGTGATGAGGCGAATGGCAAGTGT  
GCGATACTATCGGTTGGTGGAAG

[Luciferase plasmids](#)

SV40-pA-5 CGC GAC TCT AGA TCA TAA TCA GCC  
SV40-pA-3 TTA CGC GAG CTC ATA CAT TGA TGA GTT TGG  
ff-luc-5 CCA TAA GCT TCA AAA TGG AAG ACG CCA AAA ACA TAA AG  
ff-luc-3 GGA TGG ATC CTT ACA ATT TGG ACT TTC CGC C  
ren-luc-5 CCA TAA GCT TCA AAA TGG CTT CCA AGG TGT ACG AC  
ren-luc-3 GGA TGG ATC CTT ACT GCT CGT TCT TCA GCA C