

Supplemental Information. Sequences and a primer table

sgRNA expression components

1. sRNAi promoter sequence:

5' -

TCGACTTCTGCAGCTCTTCCAAATCGTACACGCCACTGGGCATTGAAGTGGACTCGCCTGCAGCATGAAA
ATTATCATAATAGCTGCAGGTTTCGATCGAGCCCGGACGCTTCTGTGCTTCTTCCAAAACCCAGCTCGCC
GTTAGACTTCGGCAAGCCGCATCGACGGCTTCCCGATCGCTTTCGGCCATGACTCGCTCGTGAACACACA
TATCCGTCGACTGCTTAAACACAATGCTAGAGCCCCGCTTCCACCAGCTCCGCGACCGTCCGAGATCGG
TCCCATGGGAGGTTTTTGTAAATTGGCGGTATAGACCTTTCGAGGCTTCTTGCGATTGGGCGAGCCATTG
CTGGTAATGTGGGTGATGGCAGTGACGTTTACATCCCCGCTACTGCCCGCAACCGGAACGTTTTCAATAT
CCCCGTCAGTGTCCATTGGCAAGATGTTTTCTTCTGGTGCCGTTGTAGTTCTTGCGACCGGTACGCTAA
AACTGTCGCGAGTTCTTCCATAACGTGATTCATTTCCGGAACGGTTCGAGTGCAATACACGAGCTTACCC
GCCGACGGATTCGCAAACCTGGTAGGAAGTAATCAGCGATAGCAAACATACGGTTTTTCCCGTTCGCGTA
GGCATCTCCAGGAGACAGTGCCACCTGCGTCCAGCGACTGTTTCAGTGCTCGCATGTACTGATGCTGCT
CAAGGTAGATGCGATCGTACGGAAAGAACACATCCAGGCCGTCCAAATCGAATCTCATTTTGACGCCTT
TTTCACACCTTCGCTTATGCAAAGCTTTCAGGAATTGCCTATGAAGAGAACGGTCAATTCCTCATTGATG
ACTGACAAGACTTGTCTGTTTACGGTAATGAAGTCTCTCGCAATCCTTTGCAATAGATCGATCGATCG
ACAGAAATGGAAGCAACTTTTGATTACTTACTGTTACAGTTATTGTGTATAGGTATACTCTTTCTATTTATAT
ATTTGCAGAAAATCATAGTTTTG - 3'

underlined = TATA box (predicted)

2. sRNAi terminator sequence:

5' -

TTTTTGCTTTTGACCGAAAGCTATCTTACTTACTACCTACCCAGCCACCGACAGCCTGTTTTCTATAGTAC
TGGAGCAAGACCAGAGTATAGAATTTGCTGCTGTTATAGCCGTT - 3'

3. sgRNA backbone sequence (redundant spacer):

5' -

NNNNNNNNNNNNNNNNNNNNNGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTAT
CAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT - 3'

4. 2X BbsI spacer insert:

5' -

GGGTCTTCGAGAAGACCTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACT
TGAAAAAGTGGCACCGAGTCGGTGCTTTTTT - 3'

Bold = BbsI-HF recognition site

5. 2X BbsI LacZ insert:

5' -

TATATATTTGCAGAAAATCATAGGG**GTCTTC**GGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGG
CAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTT
CCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGAT
TACGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCAGTGGC
CGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACCTTAATCGCCTTGCAGCACATCCC
CCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTG
AATGGCGAATGGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTACACCCGCATATGGT
GCACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCCGCTGA
CGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTGACAG**GAAGAC**CTGTTTTA
GAGCTAGAAATAGCAA - 3'

Bold = BbsI-HF recognition site

CRISPR-Cas9 episome components

GSG-P2A Sequence:

5' -

GATGAATGGGTTCCCCGCGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAA
CTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTT - 3'

Primer List

Episome Cloning Primers	
pBR322-Amp_1	CAATGATACCGCGgGATCCAC
pBR322-Amp_2	GCGTGgATCcCGCGGTATCATTG
Cas9-2A-ShBle_1	GTATCGATAATATTCTAGCTGAGGGTACCCATGGACTATAAGGACCACGA
Cas9-2A-ShBle_2	ctggcgacgtggaggagaaccctggacctggactccggcgATGGCCAAGTTGACCACTG
Cas9-2A-ShBle_3	AACGCGCGGGGAGAGGCGGTTTTCGTATTggtctcatcgaTTCGGGGAAATGTGCGCGGAACC
Cas9-2A-ShBle_6	TTCGGGTGGGCCTTTCTGCGTTTATAggtctcacgttAAGTGCCACCTGACGTCTAAGAAACC

Cas9-2A-ShBle_7	TTGTAGTCTCCGTCGTGGTCCTTATAGTCCATGGGTACCCTCAGCTAGAAT
Cas9-2A-ShBle_RFP-1	TTCCGCGCACATTTCCCCGAAtcgatgagaccAATACGCAAACCGCCTCTCC
Cas9-2A-ShBle_RFP-2	TTAGACGTCAGGTGGCACTTaactgagaccTATAAACGCAGAAAGGCCACCCG
P2A-1	agcaggctgaagtagtagctccgctcccgtctcgcgcCTTTTTCTTTTTGCCTGG
P2A-2	tccaggctcagggttctcctccacgtcgcagcctgcttcagcaggctgaagtagtagc

sgRNA target loci	
gNR-A	<i>GTCCGCCGGTACGTCAAAGATGG</i>
gNR-B	<i>GATGAATGGGTTCGCCGATGG</i>
g51092	<i>GAGCCATCAAAGTTCCACTTGGG</i>
g24739	<i>GATGACGTCAGCGTCAGCCTTGG</i>

- PAM sequencing is bolded

Golden-gate insert primers for sgRNA spacer insert	
sRNAi_gNR-A_1	TACGgaagacAGTTTTGTCCGCCGGTACGTCAAAGAGTTTTAgtcttcGACA
sRNAi_gNR-A_2	TGTCgaagacTAAAACTCTTTGACGTACCGGCGGACAAAAGTgtcttcCGTA
sRNAi_gNR-B_1	TACGgaagacAGTTTTGATGAATGGGTTCGCCGAGTTTTAgtcttcGACA
sRNAi_gNR-B_2	TGTCgaagacTAAAACTCGCGGGGAACCAATTCATCAAAAGTgtcttcCGTA
sRNAi_g51092_1	TACGgaagacAGATAGTTTTGAGCCATCAAAGTTCCACTTGTTTTAgtcttcGACA
sRNAi_g51092_2	TGTCgaagacTAAACAAGTGGAAGTTTATGAGGCTCAAAAGTATCTgtcttcCGTA
sRNAi_g24739_1	TACGgaagacAGATAGTTTTGATGACGTCAGCGTCAGCCTGTTTTAgtcttcGACA
sRNAi_g24739_2	TGTCgaagacTAAACAGGCTGACGCTGACGTCATCAAAAGTATCTgtcttcCGTA

Cloning and Reaction Primers	
GG-gRNA1-F	ATTTggtctcaTCGACTTCTGCAGCTCTTCCAAATC
GG-gRNA1-R	GCAcgtctcTACAGGAATTCGCTATAACAGCAGCAAATTCATAC
GG-gRNA2-F	ATTTggtctcaCTGTCTTCTGCAGCTCTTCCAAATC
GG-gRNA2-R	GCACggtctctCTCAATCGATGCTATAACAGCAGCAAATTC

GG-gRNA4-F	ATTGgtctcaAACCTTCTGCAGCTCTTCAAATC
LacZ-GG-1	ATTGgtctcaCTGTGGCAGCTGGCACGACAGGT
LacZ-GG-2	GCACggtctctCTCAATCGATTGTCACAGCTTGTCTGTAAGCGG
LacZ-GG-3	ATTGgtctcaTGAGGGCAGCTGGCACGACAGGT
LacZ-GG-4	GCACggtctctGGTTATCGATTGTCACAGCTTGTCTGTAAGCGG
sRNAi(GG)_2	GCACggtctctAACGGCTATAACAGCAGCAAATT
Amp-2	GAGAATTCTTGAAGACGAAGGGCC
ARS_seq	CGTGTAAGTTACAGGCAAGCG
V2_insert_2	AGCTTATCGATGATAAGCTGTC
NR-gene-1	GATGGATCAACTTAGTCTCCTCG
NR-gene-2	GCTACGATTTCTGCCATCGA
NR-KO-2*	ATTGTTTGACGGATCATGTT
NR-HRM-A	GAAGCTCGCGAAGGTGACACTCC
NR-HRM-B	TGCTTCAGAATCGCCAGCGGTG
NR-HRM-C	CCAGCGTCATCGCGCAA
NR-HRM-D	GTGGTTCGACGTTGAAGGGA
GSII_transcript_1	ATGAAATTAAACATTGCTGCTATTGCG
GSII_KO_Seq1	ACACGCACGCTTCCTGCCAA
GSII_KO_Seq4	CCTGTTGTCCGGGCATGA
GSII_KO_Seq5	CATGGATTCCGTAATGTGACG
24739_KO_1	ACAGCTGGAGATTAAATGG
24739_KO_2	ATAACACAGCCTTCTGCA
24739_KO_3	GGTGTACGCTGATATCACC
24739_KO_4	CCATCGACACGAAGCGTTAC