

Appendices

PCR programs

Table A1: InFusion PCR program

Step	Temperature	Time
1	98°C	10 sec
2	60°C	10 sec
3	72°C	30 sec
4	Go to Step 1, 34 times	
5	72°C	10 min
6	4°C	∞

Table A2: Short-range (SR; amplicon size < 1kb) and Mid-range (MIDR; for amplicon size = 1-5kb) PCR program

Step	Temperature	Time
1	93°C	3 min
2	92°C	15 sec
3	65°C	30 sec
	-1°C/ cycle	
4	65°C	2 min (SR) 4min (MIDR)
5	Go to Step 2, 8 times	
6	92°C	15 sec
7	55°C	30 sec
8	65°C	2 min (SR) 4min (MIDR)
	+20 sec/ cycle	
9	Go to Step 6, 30 times	
10	65°C	9 min
11	10°C	∞

Table A3: Recombineering PCR program

Step	Temperature	Time
1	95°C	2 min
2	95°C	30 sec
3	60°C	30 sec
4	72°C	2 min 30 sec
5	Go to Step 2, 4 times	
6	95°C	30 sec
7	72°C	2 min 30 sec
	+5 sec/ cycle	
8	Go to Step 6, 27 times	
9	72°C	7 min
10	4°C	∞

Table A4: Sequencing PCR program

Step	Temperature	Time
1	96°C	45 sec
2	92°C	10 sec
3	52°C	10 sec
4	60°C	2 min
5	Go to Step 2, 59 times	
6	10°C	∞

Primers and oligo sequences

Table A5: AAAS2 KO oligos and primers

Oligo	Sequence
CRISPR gRNA oligos (U6_gRNA (AU flip) cloning append is underlined)	
CRISPR 1	5'- <u>ACCG</u> GATTTGGTGAGGCATGGCAAA
CRISPR 2	5'- <u>ACCG</u> GACAAAAGTGAGGAGTGTGA

CRISPR 3	5'- <u>ACCG</u> GATCCCCTAAAGACCCCTGGA
CRISPR 4	5'- <u>ACCG</u> GTGAAGGCAGTTCTTGTGCCA
InFusion PCR primers (pUC19_RV InFusion cloning append is underlined)	
5F	5'- <u>GCCAGTGAATTCGAT</u> GGGAGGATTAATTTGGTACTT CCCT
3R	5'- <u>TACGCCAAGCTTGATT</u> GTAGAGAAAAGAACATGGTT TCGC
Recombineering primers (50-bp append from AAAS InFusion clone is underlined)	
U5	5'- <u>TTTGTGGAAATGGGAAACAGACCTCTGGAATCTCT</u> <u>TATACTTAGCCCAGCAAGGCGCATAACGATA</u> CCAC
D3	5' <u>TTGAAGAACACCCCAAGGTAAAGGGGTGTTGACTC</u> <u>ATTTTCCAGGATCTGCCGCCTACTGCGACTATAGA</u>
PCR primers	
F1	5'- CATGGTGTTCCTGGGAAGGACAAGG
R1	5'- GAAGTGGTAATGCCATGAGGTGGGG
F2	5'- GTCTGGCGGAGTGGAACAGCTTGG
R2	5'- GAAGTTGCAGTGAGCTGAGCACGCC
F3	5'- TCCAGCCAGCTGCCTGTGACATAGC
R3	5'- TCAGTGCCACTGCACTGGCACAATC
Sequencing primers	
Seq_F	5'- GAGATGGCACGGAATTAAG
Seq_R	5'- TCTGCAGACTGTGACCCAGG

Table A6: AAAS1 point mutation oligos and primers

Oligo	Sequence
ssODN (101 bases)	
C>A mutation oligo	5'- TACTGCCCCGTCACCAGCTCGTTATTGTGCTCATATAGGGTG ACTT <u>T</u> ACCCCGAGGCGGTGGAGGAGGGAACAACCCAGAG AGCACATCTTGCCGGTTCG – 3'

C>A + PAM mutation oligo	5'- TACTGCCCGTCACCAGCTCGTTATTGTGCTCATATAGGGTG ACTT <u>T</u> ACCCCGAG <u>A</u> CGGTGGAGGAGGGAACAACCCAGAG AGCACATCTTGCCGGTTCG - 3'
crRNA (20 base guide sequence)	5' - TATAGGGTGACTTGACCCCG... - 3'
PCR primers	
F1	5'- GCAGCACTGTTCCCTCCTCTCTGAGG
R1	5'- CTTGTCCTTCCCAGGAACACCATGG
F2	5'- AATTTGGTACTTCCCTGAATGTGGC
R2	5'- TAGTTCTCCCGCATCCTCACACTCC
Sequencing primers	
Seq_F	5'- GCGGTCTGTGCCGTTCCGGC
Seq_R	5'- TCCCTCCTCGCCCTGGCCAC

Table A7: HTT oligos and primers

Oligo	Sequence (20 base guide sequence)
CRISPR gRNA oligos (U6_gRNA (AU flip) cloning append is underlined)	
CRISPR 1	5'- <u>ACCGGCCTCCGGGG</u> ACTGCCGTGC
CRISPR 2	5'- <u>ACCGGTCGCCGG</u> CCCGCAGGCTGC
crRNAs	
crRNA 1	5'-GCCTCCGGGGACTGCCGTGC...
crRNA 2	5'-GTCGCCGGCCCGCAGGCTGC...
crRNA 3	5'-GAAGGACTTGAGGGACTCGA...
crRNA 4	5'-CTTTTCCAGGGTCGCCATGG...
InFusion PCR primers (pUC19_RV InFusion cloning append is underlined)	
F1	5'- <u>GCCAGTGAATTCGATACGCC</u> CTACCTCACCAC
R1	5'- <u>TACGCCAAGCTTGATT</u> CAGGCTGTTTTAAGTGCCAC
F2	5'- <u>GCCAGTGAATTCGAT</u> CCATTACAGTCTCACCACGC
R2	5'- <u>TACGCCAAGCTTGATA</u> CTCATTCAAACGCCTGCAG
PCR primers	

F3	5'- TCGCCACGCCTCCCTTACCATGCAG
R3	5'- CCACAACCTCATTCAAACGCCTGCAG
F4	5'- TCACACTTGGGGTCCTCAGGTCGTG
R4	5'- AACCTCCCCATCAGCAACGTGTTGG
F5	5'- TTTTACCTGCGGCCAGAGC
R5	5'- CAAACTCACGGTCGGTGCAG
Sequencing primers	
Seq_F	5'-CAGAGCCCCATTCATTGCC
Seq_R	5'-CCCAAACCTCACGGTCGGT

Table A8: Plasmid specific PCR and sequencing primers

Puro-cassette PCR primers	
Forward (EF)	5'-CATGTCTGGATCCGGGGGTACCGCGTCGAG
Reverse (ER)	5'- GCGATCTCTGGGTTCTACGTTAGTG
pUC19_RV sequencing primers	
pUC19_for	5'- AACTGTTGGGAAGGGCGATC
pUC19_rev	5'- GTTAGCTCACTCATTAGGCAC
U6_gRNA (AU flip) sequencing primers	
U6_for	5'- TCAGGAGAGCGTTCACCGAC
U6_rev	5'- CTGTTTATGTAAGCAGACAG

Plasmid maps and sequences

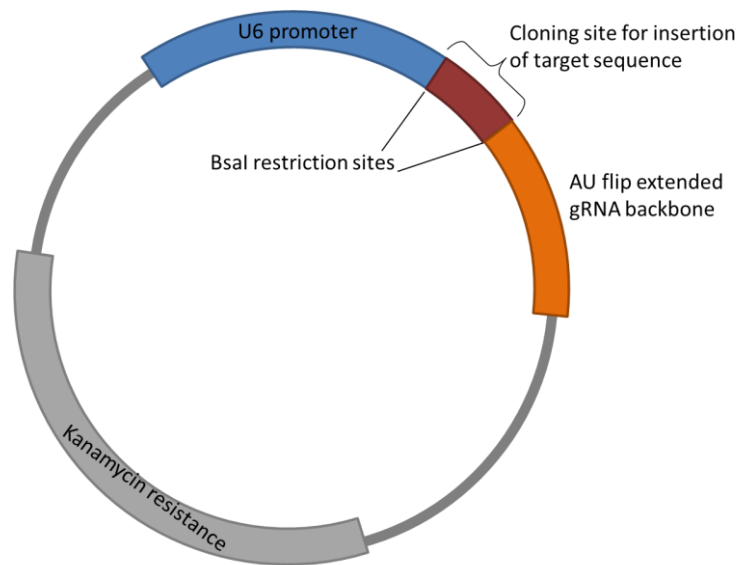


Figure A9: U6_gRNA (AU flip) expression plasmid containing extended guide RNA backbone
Sequence of U6_gRNA (AU flip) expression plasmid

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AAACGAAAGGCCAGTCTTCCGACTGAGCCTTTCGTTTTATTTGATGCCTGGCAGTTCC
CTACTCTCGGTTAACGctagcatggatctcgggccATTAACCCTCACTAAAGGGAAAGGTCCGG
CAGGAAGAGGGCCTATTTCCCATGATTCTTCATATTTGCATATACGATACAAGGCTGT
TAGAGAGATAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAAAATACG
TGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGTTTTAAAATTATGTTTTAAAT
GGACTATCATATGCTTACCCTAAGTAAAGTATTTTCGATTTCTTGGCTTTATATATCTT
GTGGAAAGGACGAAACACCGggagaccgaattcgagagggtctcaGTTTAAGAGCTATGCTGGAAA
CAGCATAGCAAGTTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAG
TCGGTGCTTTTTTTTTCATATGTGTACCTAAATtgcagctcTGGCCCGTGTCTCAAATCTC
TGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTT
ACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGC
CGCGATTAATTTCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAA
TGTCGGGCAATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAG
TTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCA
GACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTA
CTGATGATGCATGGTTACTCACCCTGCGATCCCCGAAAAACAGCATTCCAGGTAT
TAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCTCGC
CCGTTGCATTCGATTCCTGTTTGTAAATTGTCTTTTAAACAGCGATCGCGTATTTCTG
CGTCAAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGAC
GAGCGTAATGGCTGGCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCAT
TCTACCGGATTCAGTCGTCATGCTGATTTCTCACTTGATAACCTTATTTTTGACG
AGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATAACCA
GGATCTTGCCATCCTATGGAACCTGCTCGGTGAGTTTTCTCCTTATTACAGAAACGGC
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CTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTTGT

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GATAAGTCGTGTCTTACCGGGTTGGAAGTCAAGACGATAGTTACCGGATAAGGCGCAGC
GGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACA
CCGAAGTGAAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGA
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GAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTG
ACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCC
AGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTT

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Legend: Red – AU flip extended gRNA backbone
Yellow highlighted: cloning site for insertion of target sequence

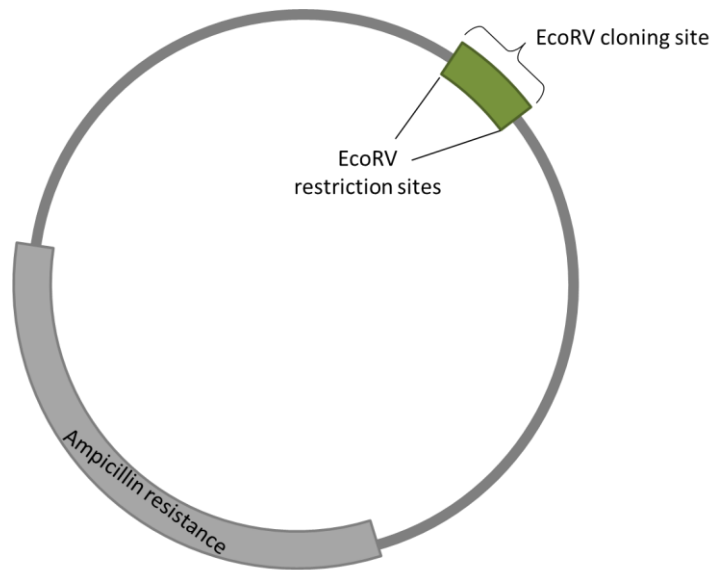


Figure A10: pUC19_RV plasmid containing EcoRV linearization site

Sequence of pUC19_RV plasmid

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TCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGT
CACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGC
GGGTGTTGGCGGGTGTCTGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTG
AGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGC
ATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGG
CCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTG
GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAACGACGGCCAGTGAATTCGAT
ATCGGCGCGCCGATATCAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCCTGTGTGAAA
TTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCC
TGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTT
CCAGTCGGGAAACCTGTCTGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAG
AGGCGGTTTTCGTATTGGGCGCTCTTCCGCTTCCCTCGCTCACTGACTCGCTGCGCTCGG
TCGTTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCAC
AGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCA
GGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGA
GCATCAGAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAG
ATACCAGGCGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCCGC
TTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCA
CGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGA

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ACCCCCCGTTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACC
 CGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAG
 CGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACAC
 TAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGA
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 CTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCG
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 AACGTTCTTCGGGGCGAAAACACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGAT
 GTAACCCACTCGTGCACCCA ACTGATCTTACGATCTTTTACTTTCACCAGCGTTTCTG
 GGTGAGCAAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGG
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 TGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTTC
 CGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGAC
 ATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC

Legend: Yellow highlighted: EcoRV linearization site for cloning

A11: Sequence of pL1L2_EF1 α -puro-polyA plasmid

CTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCTAGCATGGATCTCG
 GGGACGTCTAACTACTAAGCGAGAGTAGGGAACTGCCAGGCATCAAATAAAACGAAA
 GGCTCAGTCGGAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTGCGGTGAACGCTCTCC
 TGAGTAGGACAAATCCGCCGGGAGCGGATTTGAACGTTGTGAAGCAACGGCCCCGGAG
 GGTGGCGGGCAGGACGCCCGCCATAAACTGCCAGGCATCAAATAAGCAGAAGGCCA
 TCCTGACGGATGGCCTTTTTGCGTTTCTACAACTCTTCCTGTTAGTTAGTTACTTAAGC
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GGCCTTTTGCTCACATGTT

Legend: Yellow highlighted: attL1 and attL2 gateway sites respectively
Red: EF1 α -puro-polyA cassette

A12: Sequence of pAAAS-EF1 α -puro-polyA donor plasmid

5'-
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 HPSFNKGALLSVGWSTGRIAHIPLYFVNAQFPRFSPVLGRAQEPPAGGGGSIHDLPLFTETS
 PTSAPWDPLPGPPVLPSPHSHL

F8VZ44 (46kDa):

MKLQTQKKRLRSEDLIAEFAQVTNWSSCCLRVFAWHPHTNKFVAALLDDSVRVYNASSTI
 VPSLKHRLQRNVASLAWKPLSASVLAACQSCILWTLDPSTLSTRPSSGCAQVLSHPGHT
 PVTSLAWAPSGGRLLSASPVDAAIRVWDVSTETCVPLPWFRGGGVTNLLWSPDGSKILAT
 TPSAVFRVWEAQMWTCERWPTLSGRCQTGCWSPDGSRLFTVLGEPLIYLSLSPERCPEG
 KGCVGGAKSATIVADLSETTIQTPDGEERLGGEAHSMVWDPSEGERLAVLMKGGKPRVQDG
 KPVILLFRTRNSPVFELLPCGGIIQGEPGAQPQLITFHPSFNKGALLSVGWSTGRIAHIPLYFVN
 AQFPRFSPVLGRAQEPPAGGGGSIHDLPLFTETSPTSAPWDPLPGPPVLPSPHSHL

Legend:

Yellow highlighted: Polyclonal (Proteintech) antibody binding sequence

Red: Possible monoclonal (Sigma) antibody binding sequence

Additional figures

Genomic Location	Overlapping Gene(s)	Orientation	Query start	Query end	Length	Score	E-val	%ID
12-53307558-53307713	AAAS	Reverse	473	524	52	88.1	5e-16	100.00
12-53314297-53314443	AAAS	Reverse	182	230	49	78.0	1e-12	100.00
12-53320510-53320692	AAAS	Reverse	42	100	61	76.5	3e-12	77.05
12-53308657-53309025	AAAS	Reverse	311	382	123	75.6	7e-12	46.34
12-53321343-53321465	AAAS	Reverse	1	41	41	73.5	3e-11	100.00
12-53309156-53309281	AAAS	Reverse	271	312	42	71.7	1e-10	100.00
12-53309598-53309726	AAAS	Reverse	229	271	43	68.5	1e-09	97.67
12-53314744-53314848	AAAS	Reverse	150	184	35	58.1	3e-06	91.43
12-53308434-53308562	AAAS	Reverse	348	394	47	56.9	4e-13	80.85
12-53315332-53315457	AAAS	Reverse	93	134	42	54.2	5e-05	76.19
6-95630270-95630467	RP11-374I15.1	Reverse	390	457	68	50.9	2e-06	52.94
12-53307976-53308191	AAAS	Reverse	402	485	93	50.6	7e-04	46.24
12-53307845-53307928	AAAS	Reverse	445	472	28	49.1	0.002	100.00
12-53308217-53308354	AAAS	Reverse	393	444	52	45.6	4e-13	55.77
6-95630149-95630277	RP11-374I15.1	Reverse	451	496	46	28.3	2e-06	47.83

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