

Supplemental Table S2. Information for sgRNA evaluation

sgRNA	sgRNA target and PAM ' <u>NGG</u> ' (5'-3')	Vector whose backbone is targeted by sgRNAs	Plasmid for preparing substrate of Cas9/sgRNA cleavage	Substrate of Cas9/sgRNA cleavage	Size of substrate cleavage fragments (bps)
pOT2-G1	ACGACTCACTATAGGGAGAC <u>CGG</u>	pOT2	pOT2-CG4968 (DGRC Clone ID: LD30683)	2,670-bp pOT2-CG4968 linearized by HindIII (pOT2-CG4968/HindIII)	1,872; 798
pOT2-G4	GGCGATGATATCAGATCTGC <u>CGG</u>				1,864; 806
pOT2-G5	ATGAATCGGCTGCAGTACCC <u>GGG</u>				1,939; 731
pOT2-G9	GTTAACCTGCATTAATGAAT <u>CGG</u>				1,925; 745
pOT2-G10	TTCATTAATGCAGGTTAACCT <u>TGG</u>				1,910; 760
pOTB7-G5	CAGTAACTATAACGGTCCTA <u>AGG</u>	pOTB7, pOTB7_DraIII	pOTB7-Rpn8 (DGRC Clone ID: AT07973)	2,506-bp PstI fragment of pOTB7-Rpn8 (pOTB7-Rpn8/PstI)	1,601; 905
pOTB7-G6	CTTAGGACCGTTATAGTTACT <u>TGG</u>				1,589; 917
pOTB7-G9	ACGACGGCCAGTAACTATAAC <u>CGG</u>				1,593; 913
pFLC-G1	ATTATACGAAGTTATGGATC <u>AGG</u>	pFLC-I	pFLC-Usp5 (DGRC Clone ID: RE70722)	5,804-bp pFLC-Usp5 linearized by EcoRV (pFLC-Usp5/EcoRV)	3,540; 2,264
pFLC-G3	GCATACATTATACGAAGTTAT <u>TGG</u>				3,534; 2,270
pFLC-G6	GTATGCTATACGAAGTTATG <u>CGG</u>				3,506; 2,298
pBS-G3	TTTCGAGCTTGCGTAATCA <u>TGG</u>	pBS SK(-)	pBS-CG7747 (DGRC Clone ID: LD20635)	4,620-bp pBS-CG7747 linearized by KpnI (pBS-CG7747/KpnI)	2,806; 1,814
pBS-G3-2	TCGAGCTTGCGTAATCA <u>TGG</u>				2,806; 1,814
pBS-G5	ATTCCACACAACATACGAGC <u>CGG</u>				2,744; 1,876
pBS-G6	CTTCCGGCTCGTATGTTGTG <u>TGG</u>				2,752; 1,868
pBS-G10	CGAAATTAACCCTCACTAA <u>AGG</u>				2,835; 1,785
pBS-G13	ATGTTGTGTGAATTGTGAG <u>CGG</u>				2,764; 1,856
pCR2.1-G1	ATGTTGTGTGAATTGTGAG <u>CGG</u>	pCR2.1	pSPORT1-Prpf8 (OBS Clone ID: 9201752) ¹	1,126-bp EcoRV-HindIII fragment of pSPORT1-Prpf8 (pSPORT1-Prpf8/EcoRV+HindIII)	754; 372
pLX304-CMV-G1	GAGCTCTCTGGCTAACTGTC <u>GGG</u>	pLX304	pLX304-USP8-V5 (OBS Clone ID: 101919382) ²	2,666-bp EcoRI fragment of pLX304-USP8-V5 (pLX304-USP8-V5/EcoRI)	1,872; 794
pLX304-CMV-G7	TCTATATAAGCAGAGCTCT <u>TGG</u>				1,884; 782

pLX304-Ori-G1	<u>ACTCGCTGCGCTCGGTCGTTCCGG</u>	pLX304	pLX304-USP8-V5 (OBS Clone ID: 101919382) ²	7,278-bp PstI fragment of pLX304-USP8-V5 (pLX304-USP8-V5/PstI)	4,331; 2,947			
pLX304-Ori-G2	<u>TGCGCTCGGTCGTTCCGGCTGCGG</u>				4,325; 2,953			
pLX304-Ori-G3	<u>GCTCACTGACTCGCTGCGCTCCGG</u>				4,339; 2,939			
pLX304-Ori-G4	<u>GCGCAGCGAGTCAGTGAGCGAGG</u>				4,354; 2,924			
pLX304-Ori-G5	<u>GGAGAGGCGGTTTGCCTATTGGG</u>				4,379; 2,899			
pLX304-Ori-G6	<u>CTCACTCAAAGGCGGTAATACCGG</u>				4,289; 2,989			
pLX304-Ori-G7	<u>AGCGGTATCAGCTCACTCAAAGG</u>				4,300; 2,978			
pLX304-Ori-G11	<u>ACGGTTATCCACAGAATCAGGGG</u>				4,270; 3,008			
pLX304-Ori-G13	<u>GGTATCAGCTCACTCAAAGGCCGG</u>				4,297; 2,981			
pLX304-Ori-G14	<u>CGAGTCAGTGAGCGAGGAAGCGG</u>				4,360; 2,918			
pLX304-Ori-G16	<u>GGAAAGAACATGTGAGCAAAAGG</u>				4,239; 3,039			
pLX304-Ori-G17	<u>GGTCGTTCCGGCTGCGGCGAGCGG</u>				4,318; 2,960			
pCMV-SPORT6-G1	<u>TAGTGAACCGTCAGATCGCCTGG</u>				pCMV-SPORT6	pCMV-SPORT6-Ctbp2 (OBS Clone ID: 64867) ¹	6,364-bp pCMV-SPORT6-Ctbp2 linearized by XhoI (pCMV-SPORT6-Ctbp2/XhoI)	4,152; 2,212
pCMV-SPORT6-G2	<u>CTAGAGTCCGGAGGCTGGATCGG</u>							4,208; 2,156
pCMV-SPORT6-G3	<u>TCCGGAGGCTGGATCGGTCCCGG</u>	4,202; 2,162						
pCMV-SPORT6-G4	<u>CAGCCTCCGACTCTAGCCTAGG</u>	4,225; 2,139						
pCMV-SPORT6-G5	<u>CGGCCTAGGCTAGAGTCCGGAGG</u>	4,217; 2,147						
pCMV-SPORT6-G6-2	<u>TGTTATCCGTCCC CGGCCTAGG</u>	4,231; 2,133						
pCMV-SPORT6-G7-2	<u>CTTAGCCTAGGCCGCGGAGCGG</u>	4,236; 2,128						
pCMV-SPORT6-G8-2	<u>CGGACTCTAGCCTAGGCCGCGGG</u>	4,232; 2,132						
pCMV-SPORT6-G9	<u>ACCGGGACCGATCCAGCCTCCGG</u>	4,212; 2,152						
pCMV-SPORT6-G10	<u>ATCGGTCCCGGTGCTTCTATGG</u>	4,190; 2,174						
pCMV-SPORT6-G11	<u>ACAGCGTGGATGGCGTCTCCAGG</u>	4,159; 2,205						
pCMV-SPORT6-G12	<u>GGCGTCTCCAGGCGATCTGACGG</u>	4,148; 2,216						
pCMV-SPORT6-G13	<u>TGTCACCTAAATAGGCCTAATGG</u>	4,278; 2,086						
pCMV-SPORT6-G15	<u>AGGAAACAGCTATGACCATTAGG</u>	4,274; 2,090						
pCMV-SPORT6-G16	<u>TATGACCATTAGGCCTATTTAGG</u>	4,284; 2,080						
pCMV-SPORT6-G18	<u>GACGGATAACAATTTACACAGG</u>	4,254; 2,110						
pCMV-SPORT6-G19	<u>CTAGGCTAGAGTCCGGAGGCTGG</u>	4,213; 2,151						
pCMV-SPORT6-G21	<u>TGAAATTGTTATCCGCTCCGCGG</u>	4,237; 2,127						
pCMV-SPORT6-G23	<u>TGACCTCCATAGAAGACACCGGG</u>	4,195; 2,169						
pCMV-SPORT6-G25	<u>GGTCCCGGTGCTTCTATGGAGG</u>	4,187; 2,177						

1. Mammalian Gene Collection (MGC, <https://genecollections.nci.nih.gov/MGC/>)

2. CCSB-Broad Lentiviral Expression Library (Yang et al. 2011)