

Primer pair	Off target site (NCBI37/mm9)	Forward (5'-3')	Reverse (5'-3')
LoxP6ot 1	chr7:-60907931	ATTGTCTCCCAAGGAAGAACTC	ACAGTGACCAGAATTAGAGTAT AAGG
LoxP6ot 2	chr6:-28463696	TCTCCCTAGTCCTTCTGTGTAG	AGGCAGGAGGATCAGAAATTG
LoxP6ot 3	chr15:+72473382	CCAATCCATCTTCTGACCTCTC	ATCATGGGCAGGTTCTTATC
LoxP6ot 4	chr14:-66373202	CCATGGTTGCTGCGTTTAG	GCACTGTTTCTTTCTTCACTCC
LoxP6ot 5	chr9:+79417635	AAATGAAGGGACCTGTCTATGG	AAGTGTCAGACTTCCCAGTTAT
LoxP6ot 6	chr4:-115312664	CAGTGATGAGGGTCTAAACAGG	GCTTCCATTATGATGTGCTGAAG
LoxP6ot 7	chr9:-64594834	GTCAAAGCAGAGCTCACTAGAA	ATACTACTGCCTCTAGCCCAT
LoxP6ot 8	chr15:-99901206	CAGGTTGGCTCAACTCTTGAT	CTGTTGTCTGCCCTCCATTT
LoxP6ot 9	chr9:-34806732	GCCTCTACTCTGTCTTGCTCTA	TGCGGATGCAGTCTTGTTATAC
LoxP6ot 10	chr13:+10377580 7	ACCAACTGTAATGGGATCTGAC	CAGAGATAGGAGAACACACAGC
LoxP6ot 11	chr9:+89702030	CTTCATAGCTCCTCCCAATCAG	GAGAGTCAGGCAATACCAAAGA
LoxP6ot 12	chr17:+30398181	AATGCAAGCACTCCATCAAATC	TCTTGTCTCAAGTGTCAAAGG

Table S7. Primer pairs to assess off target (ot) Cas9 activity during CRISPR-Cas9 insertion of loxP6.

Genomic coordinates for potential off target regions were listed in the NCBI37/mm9 build. These primer pairs were used in SURVEYOR mutation assays. Forward (F) and reverse (R) primers were abbreviated accordingly and cycling conditions using JumpStart Taq can be found in [Table S2](#).