

Primer pair	Off target site (NCBI37/mm9)	Forward (5'-3')	Reverse (5'-3')
LoxP4ot 1	chr15:+72611911	TCTATTCAAGATTAAGGTACTG GCTGGC	ACTTCATTCACAAAGGCCTGAC CA
LoxP4ot 2	chr16:+42168470	ACAGCCATATAGTGTGGTCTCA TGCA	GCAACTTGGCAATGGATTACT TGAAT
LoxP4ot 3	chr7:+28954978	TGAAGCCAGGTAAGGACCACG G	GGACCAAGGTATCGTTGACAG G
LoxP4ot 4	chr4:+117809504	CTCTTGAGAAGACCGTAGTCC AATCCT	GGAGGGCTTCATACCTTGCTCT GTATA
LoxP4ot 5	chr3:+67813766	TAAGGTACTGGGGGAGGCAGT TCA	TTCCGGGAGCTGAACACAAGTG
LoxP4ot 6	chr4:+138451382	ACTCCTGAGCTGAGAAACCAA GACG	GAGCCAGTGTCTTCAAGCTGT TTT
LoxP4ot 7	chr16:+35402029	CAATGGCTTCCAGACACCAGT GTAC	GTTCACACCTTCATCCCATTCCC
LoxP4ot 8	chr11:-96986362	TGGGCAGCTGGCATTCTGTG	TAGGGTAGCAGGTGTTCCGAGA GG
LoxP4ot 9	chr16:+67621197	AGATGCCAGCCTTGACACAGG A	GGTGCCGATTGGCTGAATTCTT
LoxP4ot 10	chr11:-34085573	ACTTGATCTATGTCCATGCTAA GAGTGATG	TCCTGTATGCTTTACTGGGGTTC CT

Table S5. Primer pairs to assess off target (ot) Cas9 activity during CRISPR-Cas9 insertion of loxP4.

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](#).