

Primer pair	Off target site (NCBI37/mm9)	Forward (5'-3')	Reverse (5'-3')
LoxP5ot 1	chr1:+6805634	ACTTGATCTATGTCCATGCTAA GAGTGATG	GACAAAATCCAACACCCATTCA TGAT
LoxP5ot 2	chr14:+61414767	CTTCAAGCCTGAAGACAGAAT GAAAAGA	CAAAAACCAGTCTCTATGTTCA ATCAGGAT
LoxP5ot 3	chr3:-95805557	ATCTATTTAGGGACTTTGATGA CTCACAGC	CAGTCTATCCATTTTTAGAGCTG GGCA
LoxP5ot 4	chr1:-20708183	ATGTACCATTTAGATGTCTGTC AGGAACAC	GACAGAAACTCAGTTGGGCTAG TCTGA
LoxP5ot 5	chr1:+65426077	TGAACATTGGTTTTGGTTCCCA G	CAGCTCTCAGGACCTACCCATA CTTGT
LoxP5ot 6	chr16:-36807690	Too repetitive to design primers	Too repetitive to design primers
LoxP5ot 7	chr5:-100040548	TGCTCCATCTGACAGTCAAGGT AGG	GCAGGCCTGCCTGGAAGTTATG
LoxP5ot 8	chr14:+40469702	CTCTCTCTCTCCTCCCTACCTCT CCAC	GGTAGCCTGCTGGCTAGTATGA GAGG
LoxP5ot 9	chr4:-83614080	G TTCCTTATTTCCCGATTTTCA GAATAGG	AAGTTGCAATGGAGGACATCCC A
LoxP5ot 10	chr11:+113188213	AGTTCCCAAGTGTAAGCTCTTC GGA	TGTGTTTGGCCTCCAGTCTGTCC

Table S6. Primer pairs to assess off target (ot) Cas9 activity during CRISPR-Cas9 insertion of loxP5.

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](#).