



Targeting vector sequence

1) 5' homology long arm (4,5 kb) start 115647448 to 115651966 (NC_000076.7 of GRCm39 assembly)

CGTGAGTTGCTATTACT.....CCGACGGTATACGTTGGTG

2) Floxed fragment 1969bp (genomic seq 81-1927, Exon1-2 1299-1678=378bp (E1 66bp, Intron1 141bp, E2 177bp, intron2 252bp)) with 5'lox and 3'ftr sequences

GGCCGGCCAAGCTTCTCGAGCTTAAGGTCGACCTGCAG~~ataacttcgtataatgtatgtatagcgaagttatTTAATTAACAGGGCT.....TCAAGTTCTCTGACACCGGTGgaagttcctattctctagaa~~

~~agtataggaacttc~~ and PGK-Neo^r region 1917bp with ftr and lox sequences

gcgcccaattctaccgggtagg.....gaagctcgataccgctcgaggaagttcctattctctagaaagtataggaa~~cttcg~~cgccggg~~ataacttcgtataatgtatgtatagcgaagttat~~ggatccatcgacccctgcag

3) 3' homology short arm (3,5 kb) end : 115653820 to 11567309 (NC_000076.7 of GRCm39 assembly)

ATAAACATTTCCGAATGTTTTCATAT.....CTAATTGCTTTAATTGGAACGTATGATCCTTCTAGGAAATTAGCAATTTGT

Figure S1. Strategy for homologous recombination of the Tspan8 gene

A. A fragment of gene comprising 1.2kb of promoter, Exon1 and 2 (ATG translation initiation site is in Exon2) and a short Intron 2 sequence is replaced by the same floxed sequence containing a resistance gene flanked by FRT sites. Removal of the resistance gene generates a conditional knockout sequence. Removal of the LoxP flanked sequence generates the gene knockout.

B. A vector was built comprising 5' and 3' homology arms around the floxed region

C. Sequences of the plasmid construct. Start and end numbers refer to Genome Data Viewer <https://www.ncbi.nlm.nih.gov/genome/gdv/> (NC_000076.7 of GRCm39 assembly). Natural genomic sequences are indicated in bold. FRT and LoxP sequences are in green and pink small italic characters.