

EDG-1 KO genotyping:

Cut the tip of the tail, less than 2 mm long, lyse it at 60°C for at least 3 hours or usually o.n., in 100 µl of

PCRbuffer 10x	100 µl
MgCl ₂ 25mM	100 µl
1% gelatin	100 µl
NP40	4,5 µl
Tween20	4.5 µl
Proteinase K 20 mg/ml	60 µl
dH ₂ O up to 1000µl	

After lysis, inactivate the proteinase K by heating 10 min at 95°C.
I normally use 5 µl of this tail DNA in a 50 µl final volume PCR reaction.

Primers:

neo290

5' TCGCCTTCTTGACGAGTTCTTCTGAG

5288R

5' TGCTGCGGCTAAATTCCATG

4900F

5' CCATCCTCTACTGCAGGATCT

I used equal amounts of 3 primers in the PCR reaction mixture.

WT allele gives a 400 bp band and the KO allele, a 250 bp band. Sometimes, when it is a heterozygote sample, the WT band is very weak or absent.

PCR protocol:

94°C 10 min

94°C 1 min

55°C 1 min 45 cycles

72°C 2 min

72°C 7 min

ALTERNATIVE PROTOCOL

Two separate PCR reactions: 1 for the WT allele (**primers #1 and #2**) and a separate one for detecting the KO allele (**primers #2 and neo 3666**)

Primers:

#1 5' TAG CAG CTA TGG TGT CCA CTA G

neo 3666 5' CAT AGC GTT GGC TAC CCG TGA

#2 5' TGC TGC GGC TAA ATT CCA TG

wt allele 360bp

mutant allele 1000bp

PCR protocol:

94oC 10 min

94oC 1 min

55oC 1 min 45 cycles

72oC 2 min

72oC 7 min