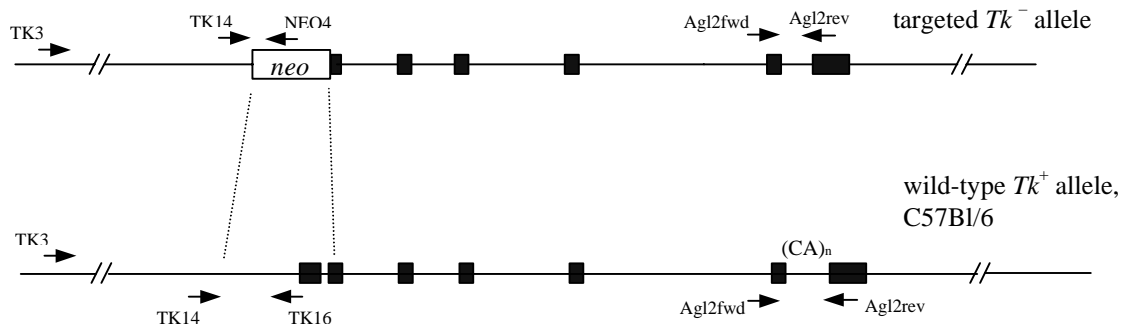
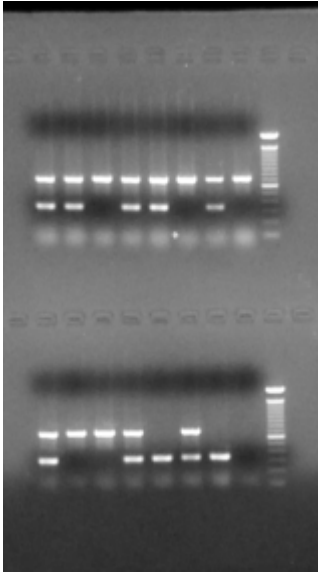


## Genotyping $Tk^{+/-}$ mice.

Tails and toes are clipped at the age of 7-10 days old. DNA is extracted from 2-3mm pieces of tails using DNeasy Tissue Kit (Qiagen). Tails are digested in 200 $\mu$ l and the DNA is eluted in final volume of 200 $\mu$ l. Of this volume, 1 to 2 $\mu$ l is used in a PCR. For PCR we use the HotStarTaq kit (Qiagen). PCR is performed with three primers TK14, TK16 and NEO4 (1 $\mu$ M each final concentration in reaction), 0.2mM of each dNTP and 1 unit of HotStarTaq and we also use 1/5 volume of Q-solution from the kit. The standard volume of PCR reaction is 20 $\mu$ l, and the temperature profile is 15min@95°+ (1min@95° + 1min@64° + 3min@72°) $\times$ 30 + 2min@72°. Usually, 5 $\mu$ l of PCR mix on 1% agarose gel produces sharp bands for targeted (~350bp) and/or wild-type alleles (~700bp).



In successful three-primer PCR, the offspring from  $Tk^{+/-}$  parents may be identified as wild-type, heterozygous and knockout ( $Tk^{-/-}$ ).



The shorter fragment on the gel corresponds to the targeted allele and the longer fragment corresponds to the wild-type allele.

Primers:

NEO4 GGAGAACCTGCGTGCAATCCATCTT

TK14 CTTGTAAGTGTGTAGCTGCCTCGAG

TK16 GGTGCAAGGCTGGGGGTCCTT

References:

Dobrovolsky et al. 1999. Environ Mol Mutagen, 34:30-38