

PCR Genotyping of *Slo*^{-/-} (*Kcnma1*^{-/-}) Mice

Breeding Tips: We generally transfer two *Slo* heterozygous (*Slo* het) breeding pairs. The *Slo* KO mice do not breed well or at all. From het crosses, *Slo* KO progeny are usually produced with normal Mendelian ratios (25% WT, 50% het, and 25% KO).

The *Slo* KO mice have some behavioral phenotypes that may affect your experiments such as ataxia, tremors, eye inflammation, low body weight, and disrupted circadian rhythms. They may have trouble getting to the food or may be barbered by littermates. At weaning, keeping the *Slo* KO pups in with the dam an extra week helps with viability and weight gain. Putting wetted food on the cage bottom and providing them a nestlet to weanlings also seem to help in some colonies. We generally separate the robust WT littermates from *Slo* KO and hets at weaning to reduce competition and stress.

Genotyping Disclaimer: We have been using **Transnetyx, Inc.** for genotyping for almost 10 years and no longer provide technical support for the following PCR protocol. If you wish to have the assays for this or other transgenic lines transferred to your account, please let me know.

Primers:

For *SLO* KO mice:

KO band: ~ 1 kb

Neo 5' 5' ATA GCC TGA AGA ACG AGA TCA GC 3'
RA 14025 3' 5' CCT CAA GAA GGG GAC TCT AAA C 3'

WT band: 332 bp

Exon1 5'-3 5' TTC ATC ATC TTG CTC TGG CGG ACG 3'
WT 3'-2 5' CCA TAG TCA CCA ATA GCC C 3'

Reaction conditions:

500ng genomic DNA (or 2µl supernatant from phenol-chloroform extraction*)
1X buffer (with MgCl₂)
0.25mM dNTPs
0.5µM each primer
2.5U Eppendorf Taq
2% DMSO
50µl reaction

Amplification conditions:

94°	2'	
94°	30s	
55° -50°	30s	X5
68°	2:00	
94°	30s	
50°	30s	X30
68°	2:00	
72°	5'	
4°	hold	

*We digest the tails in 750µl SNET + 15µl of 20mg/ml Proteinase K overnight at 55°. The next day, add 325µl phenol and 325µl chloroform; gently shake 2-5'; centrifuge at 14K at room temperature for 10'; take off 2µl of the supernatant for PCR reaction or precipitate the DNA with ethanol.

Additional tips for Slo KO PCRs:

1 Set up the PCRs as 2 master mixes.

Mix 1: H₂O, DNA, dNTPs, DMSO to 25µl

Mix 2: H₂O, buffer, and Taq to 25µl

Add Mix 2 just before beginning PCR.

2. For the most consistent results, use 2.5U/reaction Eppendorf Taq (use their standard buffer plus MgCl₂)

or

1.25-2U/reaction Fermentas Taq ((NH₄)₂SO₄ buffer, which doesn't have MgCl₂, which you should add from the stock to a final conc of 2mM).

We have tried a number of Taqs and surprisingly, a large number don't work well for this reaction.

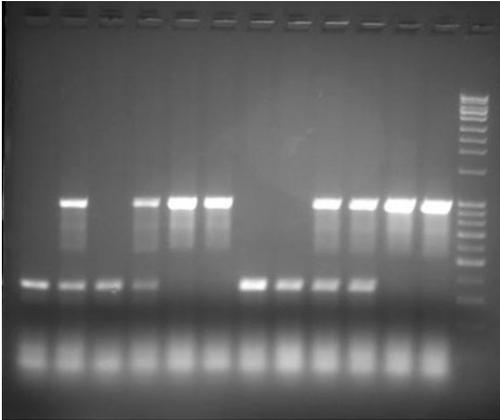
3. Volume seems to matter, use 50 µl total per reaction.

4. Run on a 1.5% gel.

5. We have seen differential amplification of bands with different amounts of DNA. Take care to cut tail pieces the same size if you are performing the PCR from non-quantified supernatants. Additionally, too much DNA will inhibit the reaction. Always ppt and spec DNA for reactions if you suspect this is a problem. We just do this so reaction routinely that we can cut corners, which is why we offer the protocol from supernatant DNA as well, but it may not work in every lab. A het cross should generate normal Mendelian ratios of pups (25% WT, 50% Het, 25% KO).

6. Some labs have noted repeated freeze/thaws of the primers causes a gradual decrement in band intensity or non-amplification (especially IDT primers). We

make small working dilution aliquots of primers for 5 reactions to avoid repeated freeze/thaw of the same aliquot.



KO/WT primers for Slo KO genotyping:

1. WT
2. Het
3. WT
4. Het
5. KO
6. KO
7. WT
8. WT
9. Het
10. Het
11. KO
12. KO