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

**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.

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

The Slo KO mice have some behavioral phenotypes that may affect your experiments, such as ataxia and disrupted circadian rhythms. Keeping the Slo KOs 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 also seem to help in some colonies. We generally separate the robust WT littermates from Slo KOs and hets at weaning to reduce competition and stress.

**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 $MgCl_2$)
0.25mM dNTPs
0.5μM each primer
2.5U Eppendorf Taq
2% DMSO
50μl reaction

**Amplification conditions:**

| | | |
|---|---|---|
| 94$^o$ | 2’ | |
| | | |
| 94$^o$ | 30s | |
| 55$^o$ -50$^o$ | 30s | X5 |
| 68$^o$ | 2:00 | |
| | | |
| 94$^o$ | 30s | |
| 50$^o$ | 30s | X30 |
| 68$^o$ | 2:00 | |
| | | |
| 72$^o$ | 5’ | |
| | | |
| 4$^o$ | hold | |

*We digest the tails in 750μl SNET + 15μl of 20mg/ml Proteinase K overnight at 55$^o$. 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: $H_2O$, DNA, dNTPs, DMSO to 25μl
Mix 2: $H_2O$, 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 $MgCl_2$)
or
1.25-2U/reaction Fermentas Taq ($(NH_4)_2SO_4$ buffer, which doesn’t have $MgCl_2$, 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.

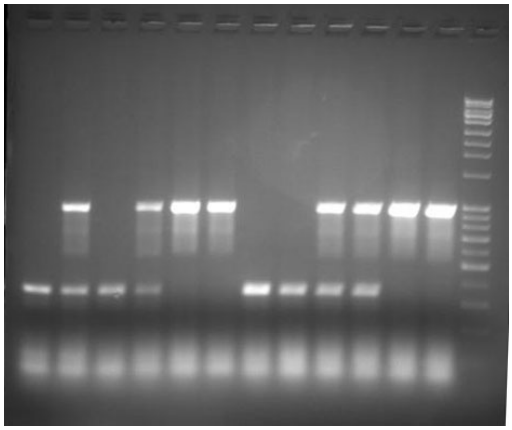
**3.** Volume seems to matter, use 50 μl total per reaction.

**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