Sex genotyping of mice

Cohn Group, GUDMAP Consortium

The sex genotyping method used by the Cohn lab is based on the highly homologous SMC genes found on the X (X chromosomal SmcX = Kdm5c) and Y (Y chromosomal SmcY = Kdm5d) chromosomes.

Homologous pair SmcX/SmcY

- SMCX-1 5'-CCGCTGCCAAATTCTTTGG-3'
- SMC4-1 5'-TGAAGCTTTTGGCTTTGAG-3'
- PCR product: females a single band (350 bp), males 2 bands (350 & 300 bp) because of an intron difference between the X and Y genes (Agulnik 1997, PMID 9060413) adapted from Case Western Transgenic Facility.

DNA extraction

1. Add tissue, forelimb of embryo, to 300µl of 25mM NaOH.
2. Heat at 95ºC for 2 hours.
3. Add 300µl 40mM Tris HCl.
4. Store at 4ºC till genotyping.

PCR mix

1. Set up the PCR mix as follows:

16.25µl	H_20
5µl	5X Green GoTaq Reaction Buffer (Promega cat #M791B)
1µl	Primer X (CCGCTGCCAAATTCTTTGG, Integrated DNA Technologies)
1µl	Primer Y (TGAAGCTTTTGGCTTTGAG, Integrated DNA Technologies)
0.5µl	dNTP mix ~ 10mM (Thermo Scientific cat #R0192)
0.25µl	*Taq* DNA Polymerase with ThermoPol Buffer (New England BioLabs cat #M0267L)
1µl	DNA

PCR Cycling Conditions

1.	94ºC	3:00 minutes	1 cycle
2.	94ºC	30 seconds	35 cycles
	54ºC	30 seconds	
	72ºC	30 seconds	
3.	72ºC	7:00 minutes	1 cycle
4.	10ºC	hold	

Analysis of the PCR products on an agarose gel

1. 5µl of PCR product is run on a 2% agarose gel.
2. Female mice are identified by a single 350bp band and males are identified by 2 smaller bands of 300bp & 350bp.

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