Genotyping of Mouse Tail DNA via PCR

I. *Mouse tailing*

[Pups are tailed (for DNA) and toed (for identification) between 8-14 days of age.]

A. Remove tail sample of approximately 0.25 inches by pinching the tip of the tail to expel blood and cutting with scissors.
B. Place tail sample in 1.5 mcf tube for digestion.
C. Scissors is washed liberally with 75% EtOH between cuts to prevent cross contamination.

II. *Tail lysis and sample preparation*

Preparation of tail lysis buffer

Final concentration [100mM Tris-HCl, pH8.8; 5mM EDTA, pH8.0; 0.2%SDS; 200mMNaCl]

- **Just prior to use, add proteinase K (Stock conc= 20mg/mL) to the buffer to achieve a final concentration of 100 ug/mL.**

A. Add 500 uL of tail lysis buffer to each tube and vortex briefly to mix.

B. Tails are incubated o/n at 55C with agitation (250rpm on a heated shaker).

C. The following morning, tubes are vortexed and centrifuged [5 min x 14000 rpm@RT] to pellet debris.

D. Dilute lysis 1:11 in sterile H_20 for use in PCR analysis.

III. *PCR*

A. Primer sequences and product size

GENE	Primer name	Primer sequence	Amplicon size
nfi-a	I2B	TGCTGTGTTCTGGTCAGTCAAG	405 bp
	I2CC	CAAAGCAAATCTCCATGCTCGG	
neo	neo57	GGAGAGGCTATTCGGCTATGAC	315 bp
	neo371R	CGCATTGCATCAGCCATGATGG	
sry (sex)	sry1	AACAACTGGGCTTTGCACATTG	166, 146 bp
	sry2	GTTTATCAGGGTTTCTCTCTAGC	(doublet)

B. PCR Reaction mixture

	COMPONENT		[STOCK]	[FINAL RXN]
1.	**10X *Taq* Buffer**		**10X**	**1X**
		Tris-HCl	100mM	10mM
		$MgCl_2$	15mM	1.5mM
		KCl	500mM	50mM
		BSA	0.2mg/mL	0.02mg/mL
2.	**Multiplex primer mix**		**10X**	**1X**
		I2B	2 uM	200 nM
		I2CC	2 uM	200 nM
		neo57	2 uM	200 nM
		neo371R	2 uM	200 nM
		sry1	2 uM	200 nM
		sry2	2 uM	200 nM
3.	**dNTP mix**		**1 mM each**	**200 uM each**
	(dA,dT,dG,dC)			
4.	*taq* polymerase		5 U/uL	0.5 Units
	(Life Technologies			
	Cat# 10966026)			
	q.s. to final volume			19.0 uL
	With H_20			
	DNA (diluted lysate)			1.0 uL
				20.0 uL rxn

C. PCR Parameters

1. 94C for 4 min (initial melt)
2. 94C, 60C, 72C x 33 cycles (amplification)
3. 72C for 9 min (final extension)

D. PCR products are separated on a 2% agarose gel, stained with EtBR, and photographed for documentation.