

PCR Protocol for Genotyping Rosa:

A. Primers:

IMR0883: AAA GTC GCT CTG AGT TGT TAT
IMR0315: GCG AAG AGT TTG TCC TCA ACC
IMR0316: GGA GCG GGA GAA ATG GAT ATG

B. Digestion of mouse tail: (Sigma-Aldrich Extract-N-Amp™ Tissue PCR Kits)

1. Add 50ul of Extraction solution
2. Add 12.5ul of Tissue Preparation Solution
3. Incubate at room temperature for 10min.
4. Incubate at 95 °C for 3min.
5. Add 50ul of Neutralization Solution B
6. Vortex for 20 sec.
7. Store at -20 °C (Digest is now ready for PCR)

C. PCR Reactants:

Biomix	10.0ul
ddH ₂ O	5.8ul
Primer 1	0.4ul
Primer 2	0.4ul
Primer 3	0.4ul
DNA template	3.0ul
Total Volume	(20ul)/reaction

*DNA is added last

*Do not add Cresol Red dye—add 5.0ul 6X Blue Loading Dye after PCR

D. PCR Conditions:

Heated Lid:	105°C	
Initial Denaturation:	94°C	1.5 min
Cycles:	35x	
	94°C	30 sec
	67°C	1 min
	72°C	1 min
Final Extension:	72°C	2 min
Final Hold:	4°C	

E. Product:

Wild-Type: 550 base pairs

Mutant: 300 base pairs

F. Notes:

1. Tails can be store at -20°C until digested
2. When genotyping embryo tails, do not add dye in the PCR reactants and reduce the DNA amount to 1.0ul