

PCR Protocol for Genotyping: ROSA

A. Digestion of mouse tail or ear notch, and **embryo tail (in red)**:

1. Add 100 μ L of Tissue Digestion Buffer and 2 μ L of Proteinase K per tail (~1-2mm length). For embryos tail add **50 μ L of Tissue Digestion Buffer and 1 μ L of Proteinase K**. Make sure tail is immersed in the buffer.
2. In a thermocycler incubate at 55°C for 1 h followed by 95°C for 8 min to inactivate the enzyme and hold at 10°C. For embryos incubate at 55°C for 30 min followed by 95°C for 8 min and hold at 10°C.
3. Vortex and store at 4°C (-20°C for long storage) or use immediately to set up the PCR.

B. PCR Genotyping Protocol: ROSA

Primers			
IMR0883	5'-	AAA GTC GCT CTG AGT TGT TAT	-3'
IMR0315	5'-	GCG AAG AGT TTG TCC TCA ACC	-3'
IMR0316	5'-	GGA GCG GGA GAA ATG GAT ATG	-3'

PCR Reaction			PCR Conditions		
BioMix (Bioline)	10.0 μ L		Heated Lid		105°C
Primer 0883	0.8 μ L		Initial Denaturation	94°C	2 min
Primer 0315	0.8 μ L		Number of Cycles	x35	
Primer 0316	0.8 μ L			94°C	30 sec
ddH ₂ O	5.6 μ L			67°C	1 min
DNA template	2.0 μ L			72°C	1 min
Total Volume	20.0 μ L		Final Extension	72°C	2 min
			Final Hold	4°C	

PCR Product Size (bp)	
Wild type band	550 bp
KO (ROSA) band	300 bp

C. Reagents

Reagent	Cat #	Final Concentration	Working Concentration
Tissue Digestion Buffer for ear notch or tail			
Tris pH8.5		50mM	
EDTA		1mM	
Tween20		0.5%	
Proteinase K (Invitrogen)	25530-015	20mg/mL	
BioMix (Bioline)	BIO-25012		
Primers			10mM