

PCR Protocol for Genotyping: PAX8 (KOES)

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

1. Add 100µL of Tissue Digestion Buffer and 2µL of Proteinase K per tail (~1-2mm length). For embryos tail add **50µL of Tissue Digestion Buffer and 1µL 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: PAX8 (KOES)

Primers			
oIMR7385	5'-	CCA TGT CTA GAC TGG ACA AGA	-3'
oIMR7386	5'-	CTC CAG GCC ACA TAT GAT TAG	-3'

PCR Reaction		PCR Conditions		
BioMix (Bioline)	10.0 µL	Heated Lid		105°C
Primer F	0.8 µL	Initial Denaturation	94°C	10 min
Primer R	0.8 µL	Number of Cycles	x35	
ddH ₂ O	6.4 µL		94°C	30 sec
DNA template	2.0 µL		63°C	1 min
Total Volume	20.0 µL		72°C	45 sec
		Final Extension	72°C	2 min
		Final Hold	10°C	

PCR Product Size (bp)	
Wild type band	
KO (PAX8) band	595 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