

PCR Protocol for Genotyping: PKD1^{neo} (Pkd1cko)

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: PKD1^{neo}

Primers			
F4 forward 1	5'-	CCT GCC TTG CTC TAC TTT CC	-3'
R5 back 1	5'-	AGG GCT TTT CTT GCT GGT CT	-3'

PCR Reaction		PCR Conditions			
BioMix (Bioline)	10.0 µL		Heated Lid		105°C
Primer F1	0.8 µL		Initial Denaturation	94°C	45 sec
Primer B1	0.8 µL		Number of Cycles	x35	
Primer 2B	0.8 µL			94°C	20 sec
ddH ₂ O	5.6 µL			56°C	25 sec
DNA template	2.0 µL			70°C	30 sec
Total Volume	20.0 µL		Final Extension	72°C	5 min
			Final Hold	10°C	

PCR Product Size (bp)	
Wild type band	180 bp
LoxP site	250 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