

PCR Protocol for Genotyping: PKD2KO

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: PKD2KO

Primers			
MG/5'flox11-13(A)-F	5'-	CCT TTC CTC TGT GTT CTG GGG AG	-3'
MG/5'flox11-13(B)-R	5'-	GTT TGA TGC TTA GCA GAT GAT GGC	-3'
MG/3'flox11-13(D)-R	5'-	CTG ACA GGC ACC TAC AGA ACA GTG	-3'

PCR Reaction		PCR Conditions		
BioMix (Bioline)	10.0 µL		Heated Lid	105°C
Primer (A)	0.8 µL		Initial Denaturation	94°C 2 min
Primer (B)	0.8 µL		Number of Cycles	x35
Primer (D)	0.8 µL			94°C 30 sec
ddH ₂ O	5.6 µL			56°C 35 sec
DNA template	2.0 µL			72°C 1 min
Total Volume	20.0 µL		Final Extension	72°C 5 min
			Final Hold	10°C

PCR Product Size (bp)	
PKD2Δneo band	318 bp
Wild type band	232 bp
KO (PKD2) band	209 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