PCR Protocol for Genotyping: Pkhd1Δ3-4

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: PKhD1Δ3-4

Primers			
Loxp-F	5'-	**GGG AAG CAG AAA TTC AGG**	-3'
Loxp-C2R	5'-	**AGA TGA AGC ACG GAT CAG TGG G**	-3'
pgkNEO-R1	5'-	**GCT CAT TCC TCC CAC TCA T**	-3'

PCR Reaction			**PCR Conditions**		
BioMix (Bioline)	10.0 µL		Heated Lid		105°C
Primer F	0.8 µL		Initial Denaturation	94°C	3 min
Primer R	0.8 µL		Number of Cycles	x35	
Primer R1	0.8 µL			94°C	20 sec
$_{dd}H_2O$	3.6 µL			56°C	30 sec
DNA template	4.0 µL			72°C	35 sec
Total Volume	20.0 µL		Final Extension	72°C	10 min
			Final Hold	10°C	

PCR Product Size (bp)	
Wild type band	185 bp
Flox band	254 bp
^3-4 band	361 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