

## PCR Protocol for Genotyping: Pkhd1HA and <sup>Δ67</sup>(can be used for all lines)

### 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: Pkhd1HA and <sup>Δ67</sup>

Primers		Sequence	
Pkhd1-1F	5'-	<b>GTGGAATTAGTATGGAA CATGTGTTG</b>	-3'
Pkhd1-1R	5'-	<b>GAAATCCATATCACAGCCAAGGCC</b>	-3'
Pkhd1-2R	5'-	<b>CATTGCCTAGGAATTCGTG</b>	-3'

PCR Reaction			PCR Conditions		
BioMix (Bioline)	10.0 μL		Heated Lid		105°C
Primer F	0.8 μL		Initial Denaturation	94°C	2 min
Primer R	0.8 μL		Number of Cycles	x35	
ddH <sub>2</sub> O	6.4 μL			94°C	20 sec
DNA template	2.0 μL			56°C	30 sec
Total Volume	20.0 μL			72°C	30 sec
			Final Extension	72°C	10 min
			Final Hold	4°C	

PCR Product Size (bp)	
Floxed band	<b>320 bp</b>
Wild type	<b>188 bp</b>
Mutant <i>Pkhd1</i> <sup>Δ67</sup>	<b>268 bp</b>

### C. Reagents

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