PCR Protocol for Genotyping PKD1Δneo (PKD1cond):

A. Primers:

F4: CCT GCC TTG CTC TAC TTT CC
R5: AGG GCT TTT CTT GCT GGT CT

B. Digestion of mouse tail: (Sigma-Aldrich Extract-N-Amp™ Tissue PCR Kits)

1. Add 50ul of Extraction solution
2. Add 12.5ul of Tissue Preparation Solution
3. Incubate at room temperature for 10min.
4. Incubate at 95°C for 3min.
5. Add 50ul of Neutralization Solution B
6. Vortex for 20 sec.
7. Digest is now ready for PCR

C. PCR Reactants for each Reaction:

Biomix	10.0ul
ddH_2O	4.2ul
Cresol red dye	2.0ul
Primer 1	0.4ul
Primer 2	0.4ul
DNA template	3.0ul
Total Volume	(20ul)/reaction

*DNA is added last

D. PCR Conditions:

Heated Lid:	105°C	
Initial Denaturation:	94°C	45 sec
Cycles:	35x	
	94°C	20 sec
	56°C	25 sec
	70°C	30 sec
Final Extension:	72°C	5 sec
Final Hold:	10°C	

E. Product:

Wild-Type: 180 base pairs
Mutant: 250 base pairs

F. Notes:

1. Tails can be store at -20°C until digested
2. When genotyping embryo tails, do not add dye in the PCR reactants and reduce the DNA amount to 1.0ul