PCR Protocol for Genotyping PKD2KO: (can also be used to genotype PKD2Δneo)

A. Primers:

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

*Only use the first two primers (*MG/5'flox11-13A-F* and *MG/5'flox11-13B-R*) if genotyping only for PKD2Δneo (also called PKD2cond)
*Use all three primers when genotyping for PKD2KO
*Use all three primers when genotyping for both PKD2KO and PKD2Δneo

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. Store at -20°C (Digest is now ready for PCR)

C. PCR Reactants:

1. For PKD2KO:

Biomix	10.0ul
ddH_2O	5.8ul
Primer 1	0.4ul
Primer 2	0.4ul
Primer 3	0.4ul
DNA template	3.0ul
Total Volume	(20ul)/reaction

2. For PKD2^neo:

Biomix	10.0ul
ddH_2O	6.2ul
Primer 1	0.4ul
Primer 2	0.4ul
DNA template	3.0ul
Total Volume	(20ul)/reaction

*DNA is added last
*Do not add Cresol Red dye—add 5.0ul 6X Blue Loading Dye after PCR

D. PCR Conditions:

Heated Lid:	105°C	
Initial Denaturation:	95°C	2 min
Cycles:	35x	
	95°C	30 sec
	54°C	30 sec
	72°C	35 sec
Final Extension:	72°C	10 min
Final Hold:	10°C	

E. Product:

Wild-type: 232 base pairs
PKD2KO: 209 base pairs
PKD2Δneo: 318 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