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Mouse Strain Datasheet

Strain Name

Pkd2^{R652X}

MGI Gene ID

MGI:8265267

Full Allele Name

Type of Allele

knock-in nonsense, point mutation

Human Gene (HGNC)

HGNC:9009 - PKD2

Genetic Background

C57BL/6J

Commercial Source

Stock Number

Link

Genotyping Protocol

See attachment

Strain Details

Pkd2^{R652X} is a CRISPR/Cas9-generated knock-in mouse model carrying a germline nonsense mutation in *Pkd2* corresponding to the human ADPKD-associated PKD2-R654X (p.Arg654*, c.1960C>T) pathogenic variant.

The mutation produces a truncated polycystin-2 protein lacking critical pore-forming and C-terminal regions, resulting in loss of channel assembly, primary cilia trafficking, and ciliary channel activity.

Homozygous *Pkd2*^{R652X/R652X} mice exhibit severe developmental abnormalities and embryonic lethality. Heterozygous *Pkd2*^{R652X/+} are viable and normal

Validation or publication

<https://insight.jci.org/articles/view/191419>

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PCR Protocol for Genotyping: *Pkd2R652X*

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

Primers				
Pkd2-R654X-nesF1	5'-	ccaaagaggtcagcggtttt		-3'
Pkd2-R654X-nesR1	5'-	gagaaggtggtggtgactca		-3'
PCR Reaction		PCR Conditions		
BioMix (Bioline)	10.0 µL	Heated Lid		105°C
Primers (@10 µM each)	0.8 µL	Initial Denaturation	94°C	5 min
		Number of Cycles	x35	
ddH ₂ O	7.2 µL		94°C	20 sec
			60°C	35 sec
			72°C	35 sec
DNA template	2.0 µL			
Total Volume	20.0 µL	Final Extension	72°C	10 min
		Final Hold	10°C	
PCR Product Size before restriction digestion (bp)				
Expected PCR size		330 bp (single clean band)		

Restriction Enzyme (RE) Analysis			
RE Digestion Reaction (20uL total volume)		RE Digestion Conditions	
PCR Product	10 µL	Incubate	37°C for 15 min
Ddel (10U/µL)	0.5 µL		65°C for 20 min
10X Cut Buffer	2 µL	Enzyme	Ddel (NEB R0175)
ddH ₂ O	7.5 µL	Run Product on	2% Agarose gel
Total Volume	20 µL		
Expected PCR Products Size after RE			
Wild Type	292 bp + 38 bp		
Mutant (R652X)	164 bp + 128 bp + 38 bp		
Note: the 38bp band may be faint on agarose gel			

C. Reagents

Reagent	Cat #	Final 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		