Zebrafish Strain Datasheet

PKD RRC Polycystic Kidney Disease Research Resource Consortium

ACCELERATING DISCOVERY IN PKD

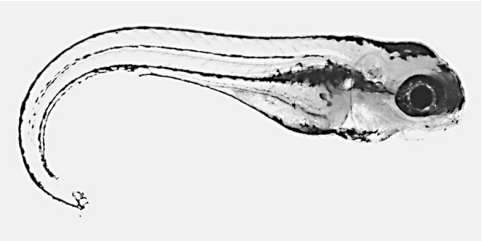
Strain Name

Nphp1 allele 2

ZFIN Gene ID ZDB-GENE-060929-1234

Type of Allele null allele

Allele Description del29; frameshift in exon 1



Human Gene (HGNC) NPHP1

Genetic Background AB

Wild type allele sequence GAA[GAGGGCCCCTTCAGATTGTCCAAAGAGAA]AC

Mutant allele sequence GAA[----------]AC

Strain Details

Homozygous mutant zebrafish are viable and fertile. Strain maintained as homozygous mutants.

Validation or publication

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Genotyping Protocol: Nphp1 allele 2

A. Digestion of tail clip:

1. Add 40 µL of 25 mM NaOH per tail. Make sure tail is immersed in the buffer.
2. Incubate at 98°C for 20 minutes.
3. Neutralize with addition of 40 µL 40 mM Tris-HCl
   a. 325 mg Tris-HCl dissolved in 50 ml sterile water.
4. Store at 4°C (-20°C for long storage) or use immediately to set up the PCR.

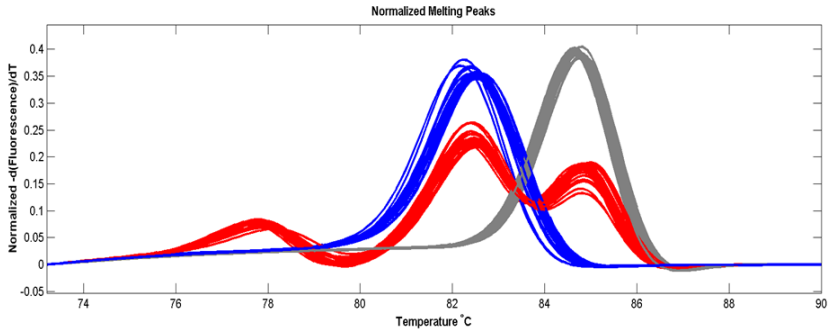
B. PCR Protocol

Primers			
Forward primer	5'-	CTGTCGCGAATACAACATGC	-3'
Reverse primer	5'-	ggtaacgtacCTGTCGCTTG	-3'

PCR Reaction		PCR Conditions		
10x Genscript Taq buffer	1.0 µL	Heated Lid		105°C
10 mM dNTP mix	0.2 µL			
15 mM $MgCl_2$	0.3 µL	Initial Denaturation	98°C	30 sec
LC Green	1.0 µL	Number of Cycles	x45	
Forward primer (10 µM)	0.3 µL		98°C	10 sec
Reverse primer (10 µM)	0.3 µL		59°C	20 sec
DNA template	1.0 µL		72°C	15 sec
$_{dd}H_2O$	5.85 µL	Final Denaturation	95°C	30 sec
Genscript Taq	0.05 µL	Final Hold	4°C	
Total volume	10.0 µL			

C. Melting Curve/HRM protocol

Following PCR, run a melt curve analysis (or HRM) with a temperature range of 65-95 °C.



Wt: gray Hets: red Homozygous: blue

D. Reagents

Reagent	Cat #	Stock Concentration	Working Concentration
Proteinase K (Invitrogen)	25530-015	20 mg/mL	
Genscript Taq (E00101)	BIO-25012		
LC Green Plus Melting Dye (Biofire Defense)	BCHM-ASY-0005		