



ACCELERATING DISCOVERY IN PKD

# Zebrafish Strain Datasheet

**Strain Name**

**ZFIN Gene ID**

**Type of Allele**

**Allele Description**

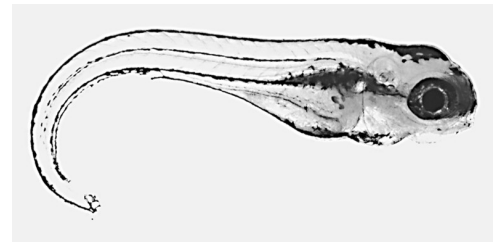
**Human Gene (HGNC)**

**Genetic Background**

**Wild type allele sequence**

**Mutant allele sequence**

**Strain Details**



**Validation or publication**

**Contact Name**

**Email**

## Genotyping Protocol:

### A. Digestion of tail clip:

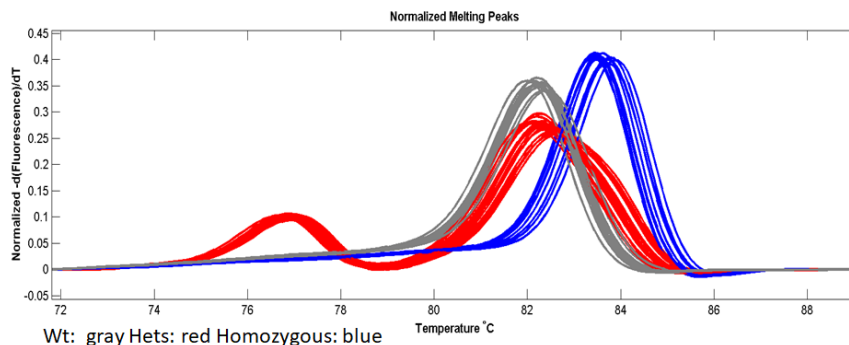
1. Add 40  $\mu\text{L}$  of 25 mM NaOH per tail. Make sure tail is immersed in the buffer.
2. Incubate at 98°C for 20 minutes.
3. Neutralize with addition of 40  $\mu\text{L}$  40 mM Tris-HCl
  - a. 325 mg Tris-HCl dissolved in 50 ml sterile water.
4. Store at 4°C (-20°C for long storage) or use immediately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

### B. PCR Protocol

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

PCR Reaction		PCR Conditions		
10x Genscript Taq buffer	1.0 $\mu\text{L}$	Heated Lid		105°C
10 mM dNTP mix	0.2 $\mu\text{L}$			
15 mM $\text{MgCl}_2$	0.3 $\mu\text{L}$	Initial Denaturation	98°C	30 sec
LC Green	1.0 $\mu\text{L}$	Number of Cycles	x45	
Forward primer (10 $\mu\text{M}$ )	0.3 $\mu\text{L}$		98°C	10 sec
Reverse primer (10 $\mu\text{M}$ )	0.3 $\mu\text{L}$		59°C	20 sec
DNA template	1.0 $\mu\text{L}$		72°C	15 sec
ddH <sub>2</sub> O	5.85 $\mu\text{L}$	Final Denaturation	95°C	30 sec
Genscript Taq	0.05 $\mu\text{L}$	Final Hold	4°C	
Total volume	10.0 $\mu\text{L}$			

### C. Melting Curve/HRM protocol



### 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		