

## FVB.129P2-*Pde6b*<sup>+</sup> *Tyr*<sup>c-ch</sup>/AntJ

Stock No: 004828

Protocol 22421: Standard PCR Assay - Generic *Pde6b*

Version 1.3

### Notes

This assay tests for the presence of the retinal degeneration *Pde6b*<sup>rd1</sup> mutation.

The WT-specific primer, oIMR2094, sits on a known SNP identified as rs47440869, located at the nucleotide indicated in red: ACC TGC ATG TGA ACC CAG TAT TCT **A**TC. Information can be found at <http://www.informatics.jax.org/snp/rs47440869>.

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

### Expected Results

Mutant = 560 bp

Heterozygote = 240 bp and 560 bp

Wild type= 240 bp

Separated by gel electrophoresis on a 1.5% agarose gel.

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR2093		AAG CTA GCT GCA GTA ACG CCA TTT		Mutant	A	
oIMR2094		ACC TGC ATG TGA ACC CAG TAT TCT ATC		Wild type	A	
oIMR2095		CTA CAG CCC CTC TCC AAG GTT TAT AG		Common	A	

#### Reaction A

COMPONENT	FINAL CONCENTRATION
ddH <sub>2</sub> O	
Kapa 2G HS buffer	1.30 X
MgCl <sub>2</sub>	2.60 mM
dNTP KAPA	0.26 mM
oIMR2093	0.50 uM
oIMR2094	0.50 uM
oIMR2095	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

#### Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.

