

STOCK *Hba^{tm1Paz} Hbb^{tm1Tow} Tg(HBA-HBBs)41Paz/J*

Stock No: 003342

Protocol 31415: Probe Assay - Pde6b<rd1> Probe

Version 1.0

Notes

Taqman qPCR protocols are run on a real time PCR instrument. Use an appropriate instrument specific Fluorophore/Quencher combination. The transgene genotype is determined by comparing ΔCt values of each unknown sample against known homozygous and hemizygous controls, using appropriate endogenous references.

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= 79 bp

Wild Type = 94 bp

>[chr5:108819412+108819505](#) 94bp TGTGGCCTCAAAGATACATCC GGATCAATACAAACCTGCACAAC

Sequence

Wt Sequence:

```
ctaccatgtcctacagcccctccaaggttataGTcactctgtggcctcaaagata
catcctTGgtggcacatcatgtctactactgttatgatgattctgtgacctgcagggttg
caggttgtattgatccagactgagggaaaaggagtaaccgtcaaagtcagcaaa
atccatattccaccagtggcatcaggacactctgcatgccaaggacagaata
```

Mut Sequence:

```
TTATAGTCACTCTGTGGCCTCAAAGATACATCCTTgaaaga
ccccgagggtggtagtcaatcaatctgaggagacctccaaggatcagcgaga
ccacgattcggatgaaacagcaagaggcttattgggaacacgggtaccgggc
gactcagtctgtcggaggactggcgccgagtggtgggttttaaccttttatagggc
tggggagcaaaaagcgt
```

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
36815		TGT GGC CTC AAA GAT ACA TCC		Common	A	
36816		TGA TCC TTG GGA GGG TCT C		Mutant Reverse	A	
36817		GGA TCA ATA CAA ACC TGC ACA AC		Wild type Reverse	A	
36818	Fluorophore-1	ACC CCC GAG GTG GGT AGT CA	Quencher-1	MUT Probe		
36819	Fluorophore-2	TGG CAC ATC ATG TCT ACT ACT GTT ATG A	Quencher-2	WT Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
36815	0.40 uM
36816	0.40 uM
36817	0.40 uM
Wt Probe	0.15 uM
Mutant Probe	0.15 uM
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	95.0	--	
2	95.0	--	
3	60.0	--	
4		--	repeat steps 2-3 for 40 cycles
5	4.0	--	Forever

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

Endpoint Fluorescence Scatter Plot

