

## B6.Cg-App<sup>em1Aduj</sup>/J

Stock No: 033013

Protocol 33278: Probe Assay - App<em1Aduj>

Version 1.0

### Notes

Taqman qPCR protocols are run on a real time PCR instrument. Use an appropriate instrument specific Fluorophore/Quencher combination.

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

Mut= 98 bp

Wt= 98 bp

### Sequence

Wt Sequence (bp changes in brackets with wt first):

CTGGGCTGACAAACATCAAGACGGAAGAGATCTCGGAAGTGAAGATG  
GATGCAGAATTC(G/C)GACATGATTCAGGAT(T/A)TGAAGTCC(GC/AT)  
CATCAAAAACGGTAGGCAAAAATAAACTGCCTCTCCCCGAGATTGCGT  
CTGGCCAGATGAAAT

G601R, F606Y, R609H

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
40451		TCG GAA GTG AAG ATG GAT GC		Forward	A	
40452		ATC TCG GGG AGA GGC AGT		Reverse	A	
41036	Fluorophore-1	TGC AGA ATT CGG ACA TGA TTC A	Quencher-1	WT Probe		
41037	Fluorophore-2	TGC AGA ATT CCG ACA TGA TTC A	Quencher-2	MUT Probe		

#### Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH <sub>2</sub> O	
40451	0.40 uM
40452	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.

