

## STOCK *Fbn1*<sup>tm3.1Hcd</sup>/J

Stock No: 025474

 Protocol 36609: End Point Analysis Assay - *Fbn1*<tm3.1Hcd> EP

Version 1.0

### Notes

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 =C/C

Heterozygote = G/C

Wild type = G/G

 >[chr2:125345838-125345923](#) 86bp CTTCTGCTGTTGTTCACTGG GGAGGATGTCTACTTACATGTGTTCA

### Sequence

GATACAGCCTGCAGCAATGAAATTGGAGTTGGTGTCTCTAAGGCT  
 TCCTGCTGTTGTTCACTGGGTAAAGCTTg/cGGAACCCCATGTGA  
 GCTGTGTCCTTCTGTGAACACATGtaagtagacatcctccatcttactactactaca  
 actactactacatatatctactgctgctgttcagtcagttct

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
49211	Fluorophore-1	AAA GCT TGC GGA ACC CCA TGT	Quencher-1	MUT Probe		
49212	Fluorophore-2	AAA GCT TGG GGA ACC CCA TGT	Quencher-2	WT Probe		
49213		CTT CCT GCT GTT GTT CAC TGG		Forward	A	
49214		GGA GGA TGT CTA CTT ACA TGT GTT CA		Reverse	A	

#### Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
49213	0.40 uM
49214	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

