

STOCK *Brca2^{tm1Brn} Gt(ROSA)26Sor^{tm3(CAG-EYFP)Hze} Trp53^{tm1Brn} Nkx3-1^{tm4(cre/ERT2)Mms} Pten^{tm1Hwu/AbshnJ}*

Stock No: 033754

Protocol 31034: Probe Assay - Pten<tm1Hwu> Probe Alternate1

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 ΔC_t 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= 139 bp

Wild Type = 173 bp

Sequence

Wt Sequence:

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cccagagctgagcatcctaagcaagcactctgcaactgagctacatcccagagtt
cataccaggatttaaggatctcaataggatagaatcaaacagatactagtaagata
aaaaccagtagtgatagaacggaagtcttgccttaGAtaatagcatcttgccctcaa
aaacttaacttgactatagagaacaaagacatcttagattcttaattcatgtg
```

Mutant

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Sequence:aaacagatactagtaagataaaaaccagtagtgatagaacggaag
tcttgccttagTGC GGCCGCACGTCTAAGAAACCATTATTATCA
TGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTT
TCGTCTTCAAGAATTCCGATCATATTCAATAACCCTTAATat
aacttgcgataatgtatgctatacgaagttaTAGGTCCCTCGAAGAGGTT
CACTAGataatagcatcttgcctcaaaaacttaactctgactatagagaaca
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JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
35320		GTT AAG TTT TTG AAG GCA AGA TGC		Common	A	
35329	Fluorophore-1	CGG AAG TCT TGC TTC TAG ATA ATA GC	Quencher-1	WT Probe		
36015		CTG AGC ATC CTA AGC AAG CA		Wild type Forward	A	
36016		CGA GGC CCT TTC GTC TTC		Mutant Forward	A	
36017	Fluorophore-2	AGG TCC CTC GAA GAG GTT CAC TAG ATA	Quencher-2	MUT Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
35320	0.40 uM
36015	0.40 uM
36016	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

