

B6;C3-Tg(Pmp-MAPT*P301S)PS19Vle/J

Stock No: 008169

Protocol 34231: Probe Assay - Tg(Pmp-MAPT*P301S)PS19Vle-Chr3 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 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= 94 bp

Wild Type = 79 bp

>[chr3:140017207+140017281](#) 75bp TCACAAACCTAAACTAGTAGCCACA CAATACTTCCATTTTGTGATC This assay is capable of distinguishing hemi from hom. Transgene insertion site is known to be on :

This assay is designed around this insertion site, and can differentiate between hemi and homs.

This assay is NOT able to be used for copy number evaluation. If this is required, it is suggested to type by qPCR.

Sequence

Wt Sequence:

agtgaacttagtggtttgtctgacatgcatcttctatcacaacctaactagtagccacagtggtgggtttTgagattttctgagTgatgacaaaatggaagtattgAagaaaaaagtctgattttactactgagctcaaaaitcAAAatagggtcaaaatcctaaitttaatgagaa
gaaatggttggttccaagaactctatcAAAatgattaagtgacctagaaaag

Mutant

Sequence:TGAACCATCACACTGGCTAGTGAACATTAGTGTGGTTTGTCTGACATGCATCTTTCTATCACAACCTAAACTAGTAGCCACAGTGTGGGTTTTcgacggatccaaaggcagcaaaaaggcagA
gaggggatactggcctggctaagcattgaaactcaagctcaaccccaattcacactcttccaacaagtcacacctcctaattagtg
ccactctggtggcctacggagatatttcaactaccaca

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
43113		TCA CAA ACC TAA ACT AGT AGC CAC A		Common	A	
43114		CTT AAG CCA GGC CCA GTA TC		Mutant Reverse	A	
43115		TCT TCA ATA CTT CCA TTT TGT CAT C		Wild type Reverse	A	
43116	Fluorophore-1	CAA AGG CAG CAA AAA GGC AG	Quencher-1	MUT Probe		
43117	Fluorophore-2	TGG GGT TTT TGA GAT TTT TCT GAG	Quencher-2	WT Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
43113	0.40 uM
43114	0.40 uM
43115	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.



