

B6;129-Postn^{tm1Jmol}/J

Stock No: 009067

Protocol 35496: Probe Assay - Postn<tm1Jmol> Probe

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

>[chr3:54367443+54367570](#) 128bp GTGAAGGAACATTCTGGAAATC TCGGAGTAGTGCTGAGTGGT

Mutant= 116 bp

Wild Type = 128 bp

Fam=Mut

Hex=Wt

Sequence

WT Sequence:

gtgaaggaacattctggaaatcaacttctaagcattgtcgtattctgactCTTcagtg
atgcctattgaccatgttatggcagcgtgggcattgtgggagccactaccactcagca
ctactccga

MUT Sequence:

GTGAAGGAACATTCTGGAAATCAACTTCTAAGCATTGTTGTTTC
GATTCTGACTCaaagccttctagggtagctctagctcgagggcgcgccccc
gctggctcttccgcctcagaag

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
12153		CTT CTG AGG CGG AAA GAA CC		Mutant Reverse	A	
46218		GTG AAG GAA CAT TCT GGA AAT C		Common	A	
46219		TCG GAG TAG TGC TGA GTG GT		Wild type Reverse	A	
46220	Fluorophore-1	CAA GCT TTC TAG GGT ACC TCT AGC TC	Quencher-1	MUT Probe		
46222	Fluorophore-2	CAG TGA TGC CTA TTG ACC ATG T	Quencher-2	WT Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
12153	0.50 uM
46218	0.50 uM
46219	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
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.

