

C;129S4-Pten^{tm1Hwu}/J

Stock No: 004597

Protocol 25050: Standard PCR Assay - Pten<tm1Hwu>

Version 7.2

Notes

Melting curve analysis is done using a Roche Light Cycler 480.

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 = 328 bp

Heterozygote = 156 bp and 328 bp

Wild type = 156 bp

Melt Peaks

Wt = 79.7°C +/- .8

Mut = 81.7°C +/- .8

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR9554		CAA GCA CTC TGC GAA CTG AG		Forward	A	
oIMR9555		AAG TTT TTG AAG GCA AGA TGC		Reverse	A	

Reaction A

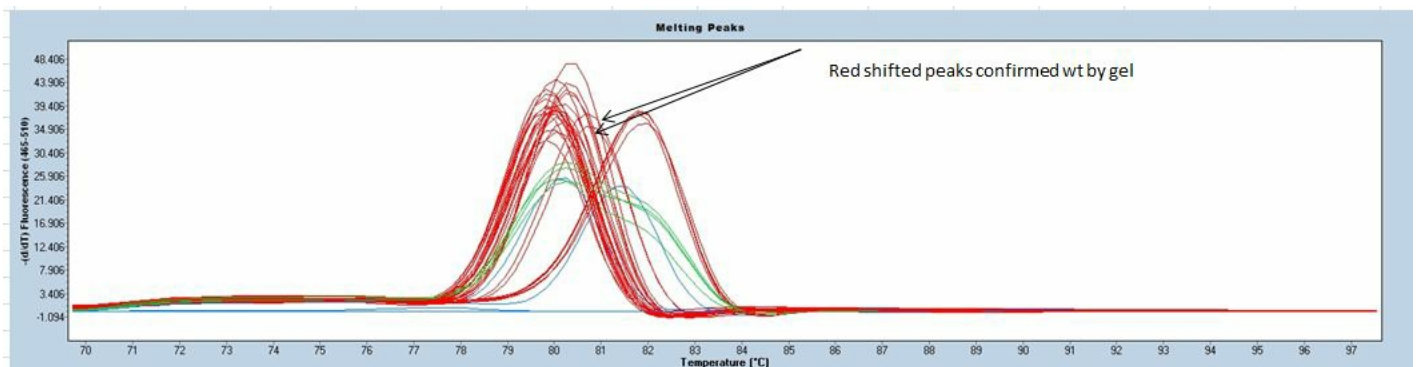
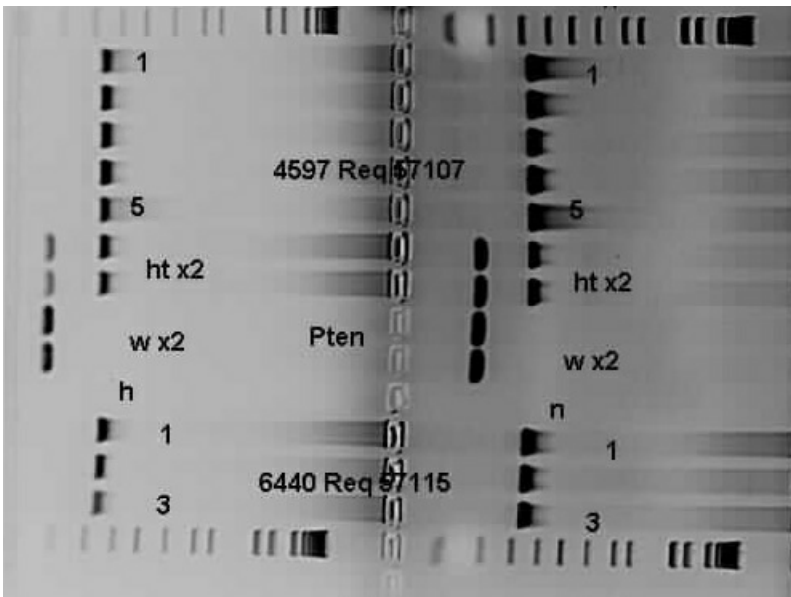
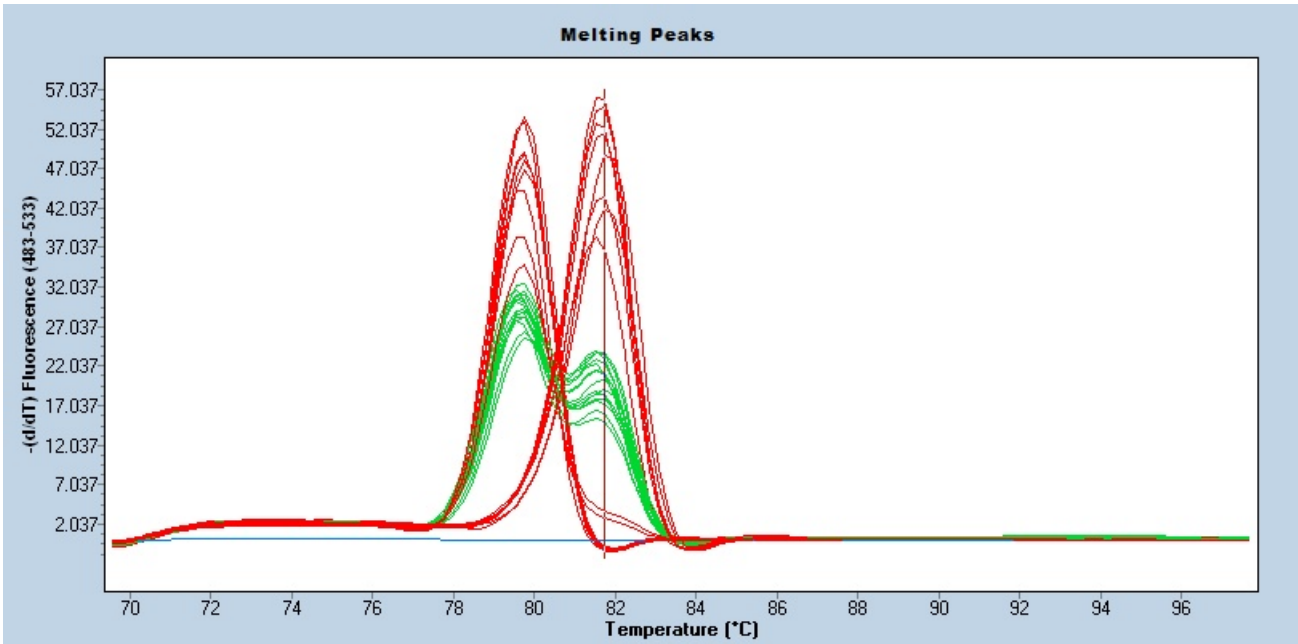
COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTPS-kapa	0.26 mM
oIMR9554	0.50 uM
oIMR9555	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

Cycling

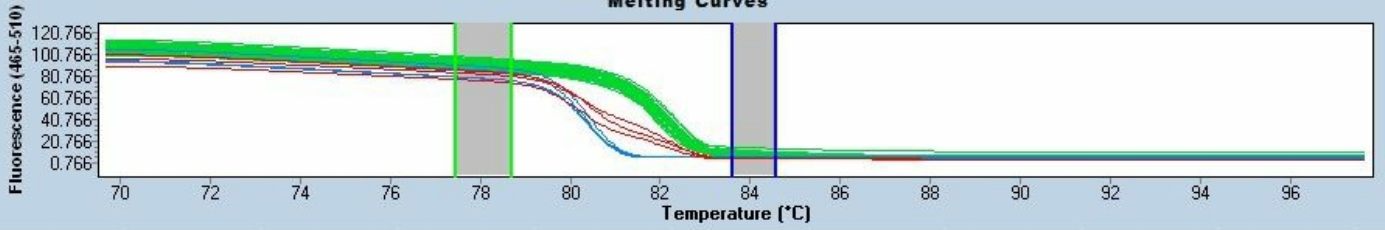
STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.



Melting Curves



Normalized Melting Curves

