

## B6.129S4-Pdgfra<sup>tm11(EGFP)Sor</sup>/J

Stock No: 007669

Protocol 26503: Standard PCR Assay - Pdgfra&lt;tm11(EGFP)Sor&gt;

Version 2.2

### 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 = 242 bp

Heterozygote = 242 bp and 451 bp

Wild type = 451 bp

Separated by gel electrophoresis on a 1.5% agarose gel.

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR7801		CCC TTG TGG TCA TGC CAA AC		Wild type Forward	A	
oIMR7802		GCT TTT GCC TCC ATT ACA CTG G		Wild type Reverse	A	
oIMR7919		ACG AAG TTA TTA GGT CCC TCG AC		Mutant Reverse	A	

#### Reaction A

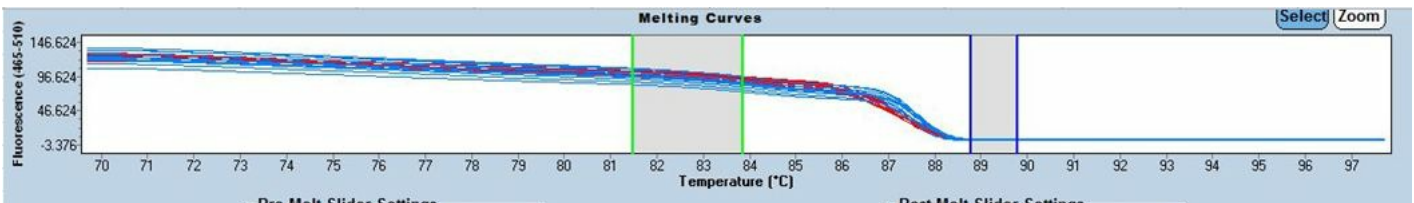
COMPONENT	FINAL CONCENTRATION
ddH <sub>2</sub> O	
Kapa 2G HS buffer	1.30 X
MgCl <sub>2</sub>	2.60 mM
dNTP KAPA	0.26 mM
oIMR7801	0.50 uM
oIMR7802	0.50 uM
oIMR7919	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

#### 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

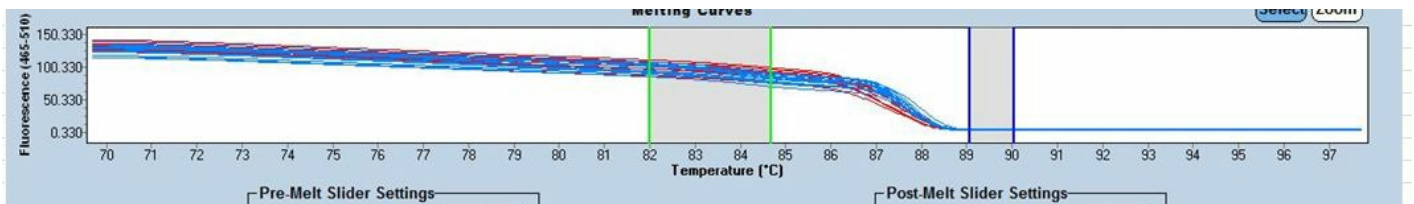
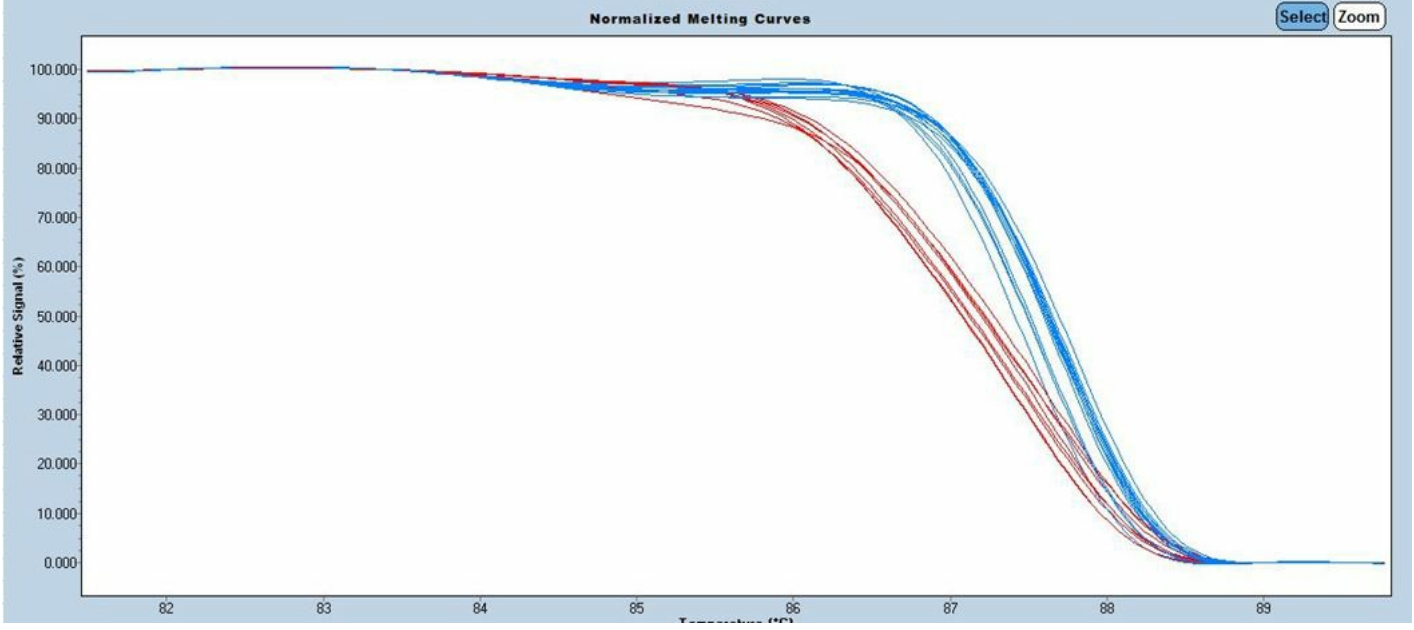
JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

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



Pre-Melt Slider Settings  
 Low 81.49 High 83.85

Post-Melt Slider Settings  
 Low 88.79 High 89.78



Pre-Melt Slider Settings  
 Low 82 High 84.68

Post-Melt Slider Settings  
 Low 89.06 High 90.05

