

STOCK *Wt1^{tm2(cre/ERT2)Wtp}/J*

Stock No: 010912

Protocol 29485: Standard PCR Assay - *Wt1^{tm2(cre/ERT2)Wtp}*>

Version 3.2

Notes

This assay does not work well without the use of a Hotstart Taq polymerase.

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 =192bp

Heterozygote = 192 bp and 400 bp

Wild type=~400bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
9650		ATC GCA GGA GCG GAG AAC		Common	A	
9651		GAA GGG TCC GTA GCG ACA		Wild type Reverse	A	
oIMR1709		GCA AAC GGA CAG AAG CAT TT		Mutant Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
9650	0.50 uM
9651	0.50 uM
oIMR1709	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.

