

B6.129S4-Cdk5r1^{tm1Lht}/J

Stock No: 004163

Protocol 30795: Probe Assay - Generic Neo

Version 3.0

Notes

PLEASE NOTE: This assay will NOT distinguish heterozygous from homozygous for anything including Tcrd<tm1Mom> mutant mice. DSW 9/13/17

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

Tg = 78 bp

IPC = 74 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
32403	Fluorophore-1	CGG CGA ATG GGC TGA C	Quencher-1	Tg Probe		
35195		GAG CGG CGA TAC CGT AAA G		Transgene Reverse	A	
oIMR1544		CAC GTG GGC TCC AGC ATT		Internal Positive Control Forward	A	
oIMR3580		TCA CCA GTC ATT TCT GCC TTT G		Internal Positive Control Reverse	A	
oIMR6773		TGG CTA CCC GTG ATA TTG CT		Transgene Forward	A	
TmoIMR0105	Fluorophore-2	CCA ATG GTC GGG CAC TGC TCA A	Quencher-2	IC Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
35195	0.40 uM
oIMR1544	0.40 uM
oIMR3580	0.40 uM
oIMR6773	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.

Endpoint Fluorescence Scatter Plot

