

B6(Cg)-Cdk15^{tm2.1Joz}/J

Stock No: 028856

Protocol 20518: Sanger sequencing Assay - Cdk15^{tm2.1Joz} Alternate1

Version 2.0

Notes

Mut = T

WT= C

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

Sequence

tagAAAATGAAGAAGTCAAGGAGACGACCTTA(ct)GAGAGC
TTAAAATGCTTCGCACTC

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
30593		CTT AAG TAG ATG ATG TTG TCA ATG G		Forward	A	
30594		GGT ACA CCG ATA GGG CAT ACT		Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTPS-kapa	0.26 mM
30593	0.50 uM
30594	0.50 uM
Glycerol	6.50 %
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.

