

C57BL/6J-Ldlr^{Hlb301}/J

Stock No: 005061

Protocol 14023: Pyrosequencing Assay - Ldlr<Hlb301>

Version 2.0

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 = A

Heterozygote = A/G

Wild Type + G

This genotyping assay uses pyrosequencing technology and is run on the Biotage
 PSQ 96MA. The Jackson Laboratory is not posting the complete details of our
 pyrosequencing genotyping assays as the primers for pyrosequencing cannot be
 used for sequencing using more traditional methods. The wild type and mutant
 nucleotides and the flanking DNA sequence are provided below.

Sequence

CACTCGCCCAAATTCACCTGCGCCT(g/a)CCCTGATGGCA
TGCTGCTGGCCAAGGAC

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
10834		CCC GTC ATC AAA TGT TGT CAG T				
10835	Fluorophore	GTC CTT GGC CAG CAG CAT				
10836		CCA AAT TCA CCT GCG				

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.00
MgCl2	2.00
dNTPS-kapa	0.20
10834	0.50
10835	0.50
Glycerol	5.00
Kapa 2G HS taq polym	0.01
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
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.