

BKS.Cg-Dock7^m +/+ Lepr^{db}/J

Stock No: 000642

Protocol 348: Restriction Enzyme Digest Assay - Lepr<db>

Version 1.1

Notes

Male mice have an unknown artifact band of ~ 170bp.

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 = 108 bp and 27 bp

Heterozygote = 27 bp, 108 bp and 135 bp

Wild type = 135 bp

Add DNA Loading Dye** to digested products and separate on a 3% agarose gel.***

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR0985		AGA ACG GAC ACT CTT TGA AGT CTC		Forward		
oIMR0986		CAT TCA AAC CAT AGT TTA GGT TTG TGT		Reverse		

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
AB PCR BufferII	1.00 X
MgCl ₂	2.00 mM
dNTP	0.20 mM
oIMR0985	1.00 uM
oIMR0986	1.00 uM
Taq DNA Polymerase	0.03 U/ul
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	52.0	--	
4	72.0	--	repeat steps 2-4 for 35 cycles
5	72.0	--	
6	10.0	--	hold***

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

Reaction B

COMPONENT	FINAL CONCENTRATION
AB PCR BufferII	X
ddH ₂ O	
RsaI	

