NOD.B10Sn-*Idd5.1*$^{C57BL/10SnJ}$/R46MrkTacJ

Stock No: 012391
Protocol 24957: Standard PCR Assay - D1Mit320
Version 2.2

Notes

THIS STRAIN NEEDS TO BE TYPED FOR 2 MIT MARKERS.

FML Buffer: 500 mM KCl, 100 mM Tris HCl pH 8.3, 15 mM MgCl2, 0.01% Gelatin

Add 42 µl of loading buffer (12% ficoll 400, 0.2% bromophenol blue, 0.04 M EDTA) diluted 1:4 with TEN (10 mM Tris pH 8.0, 1 mM EDTA pH 8.0, 10 mM NaCl) to PCR reaction. Load 5 µl on the gel. PCR products are separated on 3.5 % MetaPhor agarose gel with 0.5 x SYBR Green I Nucleic Acid Stain.

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

For JR12391: NOD is wt, C57BL/10SnJ is hom.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
D1Mit320-L		ACC AAC ACT TCC CAC AGA GG			A	
D1Mit320-R		GAA GTT GTT GGG TAG AAA CAT GC			A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTPS-kapa	0.26 mM
D1Mit320-L	0.50 uM
D1Mit320-R	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.