D2.Cg-*Utrn*^{tm1Ked} *Dmd*^{mdx}/J

Stock No: 022506
Protocol 24119: Standard PCR Assay - Utrn<tm1Ked>alternate1
Version 3.2

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 = ~310 bp
Heterozygote = 349 bp and ~310 bp
Wild type = 349 bp
Two protocols are provided because this one requires a long run on 3% gel.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
10791		CGC TTC CTC GTG CTT TAC GGT AT		Mutant Forward	A	Neo F
12402		AAG ATT TGC AGA CCG GAA GA		Common	A	
14702		TGT CAT TCT CTG AGG CCT TTC		Wild type Forward	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
10791	0.50 uM
12402	0.50 uM
14702	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

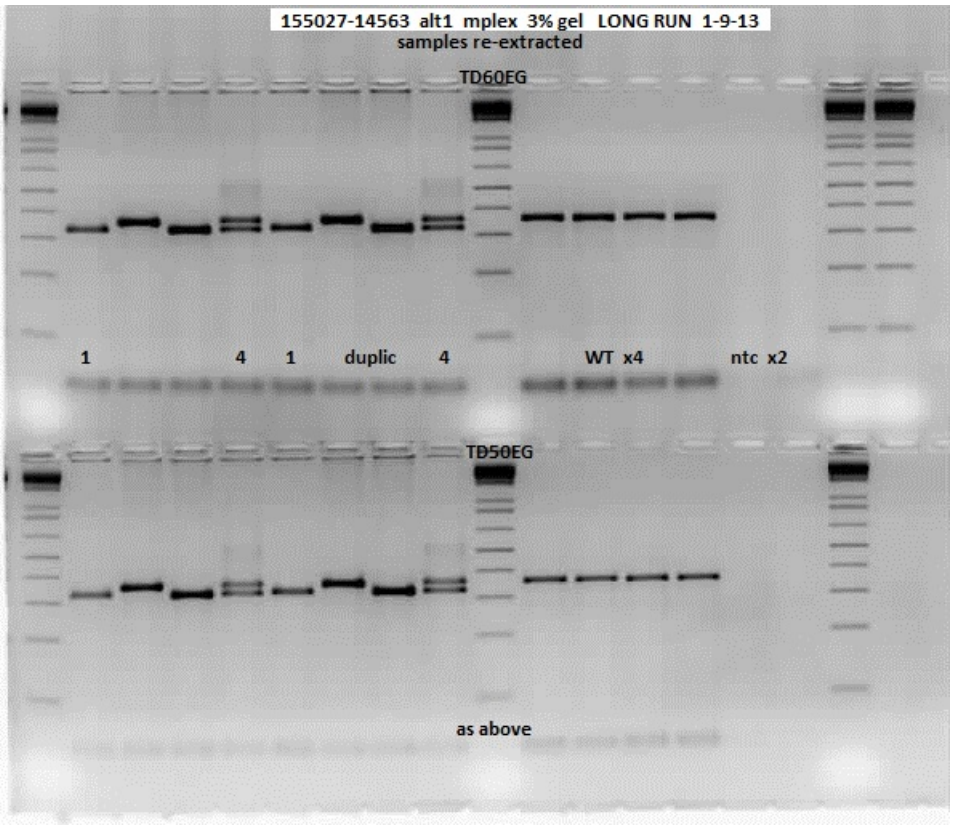
Cycling

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.


2NHET 2NHOM 2WT NTCX2
14563 UTRN
2NHET 2NHOM 2WT NTCX2


155027-14563 alt1 mplex 3% gel LONG RUN 1-9-13
samples re-extracted
TD60EG
1
4
1
duplic
4
WT x4
ntc x2
TD50EG
as above