

B6.129X1-*Fut2*^{tm1Sdo}/J

Stock No: 006262

Protocol 22543: Standard PCR Assay - *Fut2*<tm1Sdo>

Version 1.3

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 = 191 bp

Heterozygote = 191 bp and 154 bp

Wild type = 154 bp

Separated by gel electrophoresis on a 3% agarose gel.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR5304		CCT GCC ATG CTT TCT TTC CTG		Common	A	
oIMR5305		ATT CCT TCT CTG ACA GGG TTT GG		Wild type Reverse	A	
oIMR5306		TGG GTA ACG CCA GGG TTT TC		Mutant Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
oIMR5304	0.50 uM
oIMR5305	0.50 uM
oIMR5306	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.

