

B6.129S4(FVB)-Drd2^{tm1.1Mrub}/J

Stock No: 020631

Protocol 20000: Probe Assay - Drd2<tm1.1Mrub> PROBE-Alternate 1

Version 1.0

Notes

Taqman qPCR protocols are run on a real time PCR instrument. Use an appropriate instrument specific Fluorophore/Quencher combination. The transgene genotype is determined by comparing ΔCt values of each unknown sample against known homozygous and hemizygous controls, using appropriate endogenous references.

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= 165 bp

Wild Type = 77 bp

Sequence

Wt Sequence:

TGGACCAGCTGGTCAGAGAAAATGTATGTC TTCTCTACTca
cattgcaaaaaaaaaGGTCCAAGCTGGAATTctgccttgccCTGGAAT
TGTTTCAGAAGATTATACTAGAAGCAGGGCTAAATGGTGA
GAATCCCTAAGGAGGGGACAATGCCTGTGCTCCAAGGCC
TGAAACTCCTGTTGCTCT

Mutant Sequence:

GGTCAGAGAAAATGTATGTC TTCTCTACTCACATTGCAAA
AAAAAGGTCCAAGCTGGAATTaattcgatgatccggaacccttaata
actctgataatgtagctatacgaagtattaggctccctgcacctgcagcccaagctga
tcctctaattCTGGAATTGTTTCAGAAGATTATACTAGAAGCAGG
GCTAAATGGTGAGAATCCCTAAGGAGGGGACAATGCCTG
TGCT

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
29634		AAA AAG GTC CAA GCT GGA AT		Forward	A	
29635		CAC CAT TTA GCC CTG CTT CT		Reverse	A	
29636	Fluorophore-1	TGC CTT GCC CTG GAA TT	Quencher-1	WT Probe		
29637	Fluorophore-2	CCT GCA GCC CAA GCT GA	Quencher-2	MUT Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
29634	0.40 uM
29635	0.40 uM
Wt Probe	0.15 uM
Mutant Probe	0.15 uM
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	95.0	--	
2	95.0	--	
3	60.0	--	
4		--	repeat steps 2-3 for 40 cycles
5	4.0	--	Forever

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

Endpoint Fluorescence Scatter Plot

