

Stk39 (MCJB EPD0169_4_D02)

Allele: *Stk39*^{tm1a(EUCOMM)Wtsi}

Genotyping Information

These mice may be genotyped through a combination of separate PCR reactions that detect *LacZ*, the gene-specific wild type allele, and a mutant allele-specific short range PCR. Interpretation of the consolidated results produces the genotype of the mice.

For example: *LacZ* positive, mutant positive, wild type positive = heterozygous.

WARNING- WT Assay is not working properly. Please genotype using Neo qPCR or loss of allele.

➤ PCRs primer pairs and expected size bands

PCR type	Forward primer	Reverse primer	Expected size band (bp)
Mutant PCR	Stk39_34525_F	CAS_R1_Term x	153
Wild type PCR	Stk39_34525_F	Stk39_34525_R	307
LacZ PCR	LacZ_2_small_F	LacZ_2_small_R	108

➤ Primer sequences

Primer name	Primer sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Dot1l_2F	GCCCCAGCACGACCATT
Dot1l_2R	TAGTTGGCATCCTTATGCTTCATC
LacZ-RegF	GGAGTGGGATCCTTCCTGAGG
LacZ-RegR	CGCATCGTAACCGTGCATC
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG
NeoF	GGTGGAGAGGCTATTCGGC
NeoR	GAACACGGCGGCATCAG
Stk39_34525_F	CGACCGAGGCATAGTTTGTG
Stk39_34525_R	ACTGGTAGGCAGGGGACAAC
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
Dot1l_2F	GCCCCAGCACGACCATT
Dot1l_2R	TAGTTGGCATCCTTATGCTTCATC

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➤ **Reaction**

Reagent	μl
DNA (~50-100 ng)	1.0
10x Buffer	2.0
MgCl ₂ (50 mM)	0.6
PtTaq (Platinum® Taq (Invitrogen))	0.2
dNTPs (100 mM)	0.2
Primer 1 (10 μM)	0.4
Primer 2 (10 μM)	0.4
H ₂ O	<u>15.2</u>
Total	20.0

➤ **Cycling conditions**

Wild type and mutant PCRs

Cycle	Temp	Time
1	94 °C	5 min
2	94 °C	30 sec
3	58 °C	30 sec
4	72 °C	45 sec
5	Go to '2' + 34 cycles	
6	72 °C	5 min
7	12 °C	forever

LacZ PCR

Cycle	Temp	Time
1	94 °C	5 min
2	94 °C	30 sec
3	60 °C	30 sec
4	72 °C	30 sec
5	Go to '2' + 34 cycles	
6	72 °C	5 min
7	12 °C	forever

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