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# Syt1 (MDNJ EPD0214\_1\_H11)

Allele: Syt1<sup>tm1a(EUCOMM)Wtsi</sup>

## **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.

#### PCRs primer pairs and expected size bands

PCR type	Forward primer	Reverse primer	Expected size band (bp)
Mutant PCR	Syt1_42058_F	CAS_R1_Term	174
Wild type PCR	Syt1_42058_F	Syt1_42058_R	411
LacZ PCR	LacZ_2_small_F	LacZ_2_small_R	108

### > Primer sequences

Primer name	Primer sequence (5' > 3')		
CAS_R1_Term	TCGTGGTATCGTTATGCGCC		
Syt1_42058_F	ACCATTAGCAACCAGGGAGG		
Syt1_42058_R	TCATTTACACGTCGCTTGCC		
LacZ_2_small_F	ATCACGACGCGCTGTATC		
LacZ_2_small_R	ACATCGGGCAAATAATATCG		

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#### Reaction

Reagent	μΙ
DNA (~50-100 ng)	1.0
10x Buffer	2.0
MgCl <sub>2</sub> (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
$H_2O$	<u>15.2</u>
Total	20.0

## Cycling conditions

Wild type and mutant PCRs

Whatype and mutant i ONS		Lacz I OR			
Cycle	<b>;</b>		Cycle		
1	94 °C	5 min	1	94 °C	5 min
2	94 °C	30 sec	2	94 °C	30 sec
3	58 °C	30 sec	3	60 °C	30 sec
4	72 °C	45 sec	4	72 °C	30 sec
5		' + 34 cycles	5		+ 34 cycles
6	72 °C	5 min	6	72 °C	5 min
7	12 °C	forever	7	12 °C	forever

Lac7 PCR

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