

Stock Number: 007900

Strain Name: [C57BL/6-Gt\(ROSA\)26Sor^{tm1\(HBEGF\)Awai/J}](#)

Allele: Gt(ROSA)26Sor^{tm1Sor}

Gt(ROSA)26Sor^{tm1sor}

Protocol Name: STD

Method: Standard PCR

Version: 4.0

Created: 11-December -2014

Updated: 13-September-2016

Mutant = 340 bp

Expected Results: Heterozygote = 340 bp and ~650 bp

Wild type = ~650 bp

Gel Image

Image	Image file
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[View](#)

Separated by gel electrophoresis on a 1.5% agarose gel.

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Primer Type	Note
oIMR8052		GCG AAG AGT TTG TCC TCA ACC		Mutant Reverse	SA site
oIMR8545		AAA GTC GCT CTG AGT TGT TAT		Common	ROSA26
oIMR8546		GGA GCG GGA GAA ATG GAT ATG		Reverse	ROSA26

Reaction/Components A

Reaction Component	Volume (µl)	Final Concentration	Total Volume (µl)
ddH2O	4.25		4.25
5 X Kapa 2G HS buffer	2.40	1 X	2.4
25 mM MgCl2	0.96	2 mM	.96
10 mM dNTP KAPA	0.24	.2 mM	.24
20 uM oIMR8052	0.30	.5 uM	.3
20 uM oIMR8545	0.30	.5 uM	.3
20 uM oIMR8546	0.30	.5 uM	.3
5 mM 10x Loading Dye	1.20	.5 mM	1.2
2.5 U/ul Kapa 2G HS taq polymerase	0.05	.01 U/ul	.05
DNA	2.00		2

Cycling

Step #	Temp °C	Time	Note
1	94	2 min	
2	94	20sec	
3	65	15sec	-0.5 C per cycle decrease
4	68	10sec	
5			repeat steps 2-4 for 10 cycles
6	94	15sec	
7	60	15sec	
8	72	10sec	
9			repeat steps 6-8 for 28 cycles
10	72	2 min	
11	10		hold

Protocol taken from:

https://www2.jax.org/protocolsdb/f?p=116:5:0::NO:5:P5_MASTER_PROTOCOL_ID,P5_JRS_CODE:13844,007900