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Stock Number: **004781**

Strain Name: **B6.129P2-Lyz2^{tm1}(cre)Ifo/J**

Allele: Lyz2^{tm1}(cre)Ifo

Protocol Name: Lyz2^{tm1}(cre)Ifo

Method: Separated PCR

Version: 4.0

Created: 28-May -2010 Updated: 25-August -2010

Notes: Use Perfect Match© (Stratagene) added before Taq to delete non-specific bands.

Expected Results: Mutant = ~700 bp
Heterozygote = ~700 bp and ~350 bp
Wild type = 350 bp

Gel Image

Image	Image file
	View Full Size

Gel Information: Separated by gel electrophoresis on a 1.5% agarose gel.

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Primer Type
oIMR3066	-	CCC AGA AAT GCC AGA TTA CG	-	Mutant
oIMR3067	-	CTT GGG CTG CCA GAA TTT CTC	-	Common
oIMR3068	-	TTA CAG TCG GCC AGG CTG AC	-	Wild type

Number of Reactions

Reaction/Components

Reaction Component	Volume (µl)	Final Concentration	Total Volume (µl)
ddH2O	5.02	-	5.02
2.5 mM dNTP	0.96	0.20 mM	0.96
20 uM oIMR3066	0.30	0.50 uM	0.30
20 uM oIMR3067	0.60	1.00 uM	0.60
5 mM DNA Loading Dye	1.66	0.69 mM	1.66
1 U/ul Perfect Match (Stratagene)	0.20	0.02 U/ul	0.20
DNA	2.00	-	2.00
10 X KAPA Rxn Buffer	1.20	1.00 X	1.20

Cycling

Step #	Temp °C	Time	Note
1	94	3 min	-
2	94	30 sec	-
3	62	1 min	-
4	72	1 min	repeat steps 2-4 for 35 cycles
5	72	2 min	-
6	10	-	hold

5 U/ul KAPATaq DNA Polymerase 0.06 0.03 U/ul 0.06

Reaction/Components

Reaction Component	Volume (μl)	Final Concentration	Total Volume (μl)
ddH2O	5.30	-	5.30
10 X KAPA Rxn Buffer	1.20	1.00 X	1.20
2.5 mM dNTP	0.96	0.20 mM	0.96
20 uM oIMR3067	0.60	1.00 uM	0.60
20 uM oIMR3068	0.02	0.03 uM	0.02
5 mM DNA Loading Dye	1.66	0.69 mM	1.66
1 U/ul Perfect Match (Stratagene)	0.20	0.02 U/ul	0.20
5 U/ul KAPATaq DNA Polymerase	0.06	0.03 U/ul	0.06
DNA	2.00	-	2.00

Cycling

Step #	Temp °C	Time	Note
1	94	3 min	-
2	94	30 sec	-
3	62	1 min	-
4	72	1 min	repeat steps 2-4 for 35 cycles
5	72	2 min	-
6	10	-	hold

This genotyping protocol is used for the following strains:

Stock Number	Strain Name
004781	B6.129P2- <i>Lyz2^{tm1(cre)lfo}/J</i>
008882	STOCK <i>Bcl2^{tm1lrt}/J</i>

[Genotyping resources and troubleshooting](#)

This page contains guidelines, frequently asked questions, and helpful external links to genotyping and PCR resources.