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Stock Number: **003263**

Strain Name: **B6:129S2-Cdkn1a^{tm1Tyj}/J**

[Allele:](#) Cdkn1a^{tm1Tyj}

[Protocol Name:](#) Cdkn1a^{tm1Tyj}

[Method:](#) Separated PCR

[Version:](#) 2.1

[Created:](#) 15-May -2003 [Updated:](#) 19-June -2009

Notes: This assay does not work well without the use of a Hotstart (We are using Taq Start Antibody mixed 1:1 with Taq polymerase).

Mutant = ~700 bp

Expected Results: Heterozygote = 872 bp and ~700 bp

Wild type = 872 bp

Gel Image



[Image file](#)

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

Protocol Primers

Primer	5' Label	Sequence 5' --> 3'	3' Label	Primer Type
oIMR0845	-	TGA CGA AGT CAA AGT TCC ACC	-	Wild type
oIMR8294	-	AAG CCT TGA TTC TGA TGT GGG C	-	Common
oIMR8296	-	GCT ATC AGG ACA TAG CGT TGG C	-	Mutant Reverse

[Number of Reactions](#)

Reaction/Components	Reaction Component	Volume (µl)	Final Concentration	Total Volume (µl)	Cycling			
					Step #	Temp °C	Time	Note
	ddH2O	3.18	-	3.18	1	94	3 min	-
	10 X PCR Buffer II (ABI)	1.20	1X	1.20	2	94	30 sec	-
	25 mM MgCl2	1.20	2.5 mM	1.20	3	64	1 min	-
	2.5 mM dNTP	0.96	.2 mM	0.96	4	72	1 min	repeat steps 2-4 for 35 cycles
	20 uM oIMR8294	0.80	1.33 uM	0.80	5	72	2 min	-
	20 uM oIMR8296	0.80	1.33 uM	0.80	6	10	-	hold
	5 mM DNA Loading Dye	1.66	.69 mM	1.66				
	5 U/ul Taq DNA Polymerase + TaqStart AB (1:1)	0.20	.02 U/ul	0.20				
	DNA	2.00	-	2.00				

Reaction/Components	Reaction Component	Volume (µl)	Final Concentration	Total Volume (µl)	Cycling			
					Step #	Temp °C	Time	Note
	ddH2O	3.38	-	3.38	1	94	3 min	-
	10 X PCR Buffer II (ABI)	1.20	1X	1.20	2	94	30 sec	-
	25 mM MgCl2	1.20	2.5 mM	1.20	3	64	1 min	-
	2.5 mM dNTP	0.96	.2 mM	0.96	4	72	1 min	repeat steps 2-4 for 35 cycles
	20 uM oIMR8294	0.80	1.33 uM	0.80	5	72	2 min	-
	20 uM oIMR0845	0.60	1 uM	0.60	6	10	-	hold
	5 mM DNA Loading Dye	1.66	.69 mM	1.66				
	5 U/ul Taq DNA Polymerase + TaqStart AB (1:1)	0.20	.02 U/ul	0.20				
	DNA	2.00	-	2.00				

This genotyping protocol is used for the following strains:

Stock Number	Strain Name
003263	B6:129S2-Cdkn1a ^{tm1Tyj} /J

[Genotyping resources and troubleshooting](#)

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