

Genotyping protocol

General information:

Strain name	C57Bl/6 Igf-1
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Primers:

Name	Sequence	Primer type
Igf1 forw.	GTCTAACACCAGCCCATTCTGATT (5'-3')	gene-specific
Igf1 rever.	GACTCGATTTTCACCCACTCGATCG(5'-3')	gene-specific
Neo forw.	GCTTGGGTGGAGAGGCTATTC (5'-3')	selection cassette
Neo rever.	CAAGGTGAGATGACAGGAGATC (5'-3')	selection cassette

In case more than two primers are introduced, please indicate how they should be combined:

	Forward primer	Reverse primer
e.g. wt		
e.g. mut		

Reaction mix:

dH2O	35,5	μl
Buffer II, 10x	5	μl
MgCl ₂ , 25 mM	4	μl
dNTPs	1	μl
Forward primer	1	μl
Reverse primer	1	μl
Taq polimerase	0,5	μl
DNA	2	μl
Final volume	50	μl

PCR program:

94	°C	2	min	x35
94	°C	30	sec	
58	°C	45	sec	
72	°C	45	min	
72	°C	5	min	

Expected fragment size:

wt	250	bp
mutant	284	bp

Comments/Additonal information:

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