

Genotyping protocol

General information:

Strain name	129-Pik3cb<tm2.1Bvan>/Cnbc
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Primers:

Name	Sequence	Primer type
b11+5	CTTAGGGAAGAGCGAGGAGGAAG	
bseq2+3	AAGAAGTATGTACACCTCTCTGGT	

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

	Forward primer	Reverse primer
Mutant	b11+5	bseq2+3

Reaction mix:

ddH ₂ O	18,5	μl
PCR Buffer 15mM MgCl ₂	2,5	μl
dNTPs 2,5mM	2,5	μl
Primer 100μM	0,125	μl
Primer 100μM	0,125	μl
Taq Polymerase 5U/μl	0,250	μl
DNA	1	μl
Final volume	25	μl

PCR program:

94	°C	5	min	X40
94	°C	30	sec	
60	°C	30	sec	
72	°C	30	sec	
72	°C	10	min	

Expected fragment size:

WT	455 bp
HET	455bp + 605bp (+Hetero-duplex)
KI-C	605 bp

Comments/Additonal information:

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