

Genotyping protocol

General information:

Strain name	STOCK Eif2ak4<tm1.2Dron> Eif2ak2<tm1Jcbe>/Cnbc
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Primers:

Name	Sequence	Primer type
Na31	GGAAC TTTGGAGCAATGGA	
Na32	TGCCAATCACA AATCTAAAAC	
Na33	TGTTCTGTGGCTATCAGGG	
Mc Neo-2	TGACGAGTTCTTCTGAGGG	

Name	Sequence	Primer type
15 S	TCTCCCAGCGGAATCCGCACATCG	
18 AS	TGCCACTGTCAGAATCTGAAGCAGG	
4 AS	ATCCAGGCGTTGTAGTAGCGACA	

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

	Forward primer	Reverse primer
Eif2ak4tm1_WT	Na31	Na32
Eif2ak4tm1_KO	Na33	Mc Neo-2

	Forward primer	Reverse primer
Eif2ak4tm1.2_WT	15 S	4 AS
Eif2ak4tm1.2_KO	15 S	18 AS

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:

95	°C	5	min	X35
95	°C	30	sec	
58	°C	30	sec	
72	°C	45	sec	
72	°C	10	min	

Expected fragment size:

Eif2ak4tm1_WT	225	bp
Eif2ak4tm1_KO	~500	bp

Eif2ak4tm1.2_WT	374	bp
Eif2ak4tm1.2_KO	~650	bp

Comments/Additonal information: