

Genotyping protocol

General information:

Strain name	FVB.Cg-Dnajc5<tm1Sud> Tg(Thy1-GFP*)1Rfc/Cnbc
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Primers:

Name	Sequence	Primer type
PLC 30	ATGAGTAAAGGAGAAGAAC	
PLC 31	TTAACCGGTTTTGTATAG	
25938	CAAGAATGCAACCTCAGATGAC	
Oimr8574	GAGCGCGCGCGCGGAGTTGTTGAC	
25939	CTTTTAAGTGTGTTTACTTTTTGGTG	

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

	Forward primer	Reverse primer
SYPHA	PLC 30	PLC 31
CSPalpha-WT	25938	25939
CSPalpha-Mut	Oimr8574	25939

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 SYPHA:

95	°C	5	min	X44
97	°C	30	sec	
50	°C	45	sec	
65	°C	45	sec	
65	°C	5	min	

Expected fragment size SYPHA:

wt	This assay will NOT distinguish hemizygous from homozygous transgenic animals
mutant	720 bp

PCR program CSPalpha-KO:

94	°C	5	min	X35
94	°C	35	sec	
60	°C	35	sec	
72	°C	35	sec	
72	°C	3	min	

Expected fragment size CSPalpha-KO:

wt	336 bp
mutant	150 bp

Comments/Additonal information:

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