Genotyping protocol

General information:

Strain name	B6-Sdhd-KO

Primers for PCR 1 and PCR 2:

Name	Sequence	Primer type
501	GGAAGGCTCCAAGGGTGCAG	gene-specific
502	CACATACACGCAGGCACTGG	gene-specific
505	TCTCTGCAGTGGCCAAGG	gene-specific
504	ATCTTGTTCAATGGCCGATCCC	selection cassette

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

	Forward primer	Reverse primer
Wild type	501	502
Null allele	505	504

Reaction mix for wt allele detection (PCR 1):

DNA	2	µl
DreamTaq Green PCR Master Mix (2x)	12.5	µl
501 primer 20 µM	1	µl
502 primer 20 µM	1	µl
H_2O	8.5	µl
Final volume	25	µl

PCR 1 program:

95	°C	3	min	
95	°C	30	sec	X35
65	°C	30	sec	
72	°C	60	sec	
72	°C	10	min	

Expected fragment size:

wt	370	bp

Comments/Additional information:

Null allele detection requires a different PCR reaction (see below)

Reaction mix for null allele detection (PCR 2):

DNA	2	µl
DreamTaq Green PCR Master Mix (2x)	12.5	µl
505 primer 20 µM	1	µl
504 primer 20 µM	1	µl
H_2O	8.5	µl
Final volume	25	µl

PCR 2 program:

95	°C	3	min	
95	°C	30	sec	X35
65	°C	30	sec	
72	°C	60	sec	
72	°C	10	min	

Expected fragment size:

Null allele	430	bp

Comments/Additional information:

The targeting construct contains a neo cassette. If *Sdhd*$^{+/-}$ mice are crossed with CRE-expressing lines, neo cassette can be excised from the null allele and PCR-3 will be needed for genotyping (see below)

Primers for PCR 3:

Name	Sequence	Primer type
505	TCTCTGCAGTGGCCAAGG	gene-specific
506	GAGTCTATTTCAAACATGTAAGCACC	gene-specific

Reaction mix for « excised » null allele detection (PCR 3):

DNA	2	µl
DreamTaq Green PCR Master Mix (2x)	12.5	µl
505 primer 20 µM	1	µl
506 primer 20 µM	1	µl
H_2O	8.5	µl
Final volume	25	µl

PCR 3 program:

95	°C	3	min	
95	°C	30	sec	X35
58	°C	30	sec	
72	°C	60	sec	
72	°C	10	min	

Expected fragment size:

« Excised » Null allele	300 bp