

Genotyping protocol

General information:

Strain name	C57BL/6-Dbh<tm1a(EUCOMM)Wtsi>/Cnbc
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Primers:

Name	Sequence	Primer type
Dbh 5P arm Miguel_Fw	GGGGAAACTCAAACACTGCT	
Dbh 3P arm CritReg Miguel_Rv	GGAGGCAGGGGAAAGGTATT	
Dbh 5P arm Miguel_Fw	GGGGAAACTCAAACACTGCT	
Cassette_Rv	ACCACCTCATCAGAAGCAGG	

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

	Forward primer	Reverse primer
wt	Dbh 5P arm Miguel_Fw	Dbh 3P arm CritReg Miguel_Rv
KO allele	Dbh 5P arm Miguel_Fw	Cassette_Rv

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 WT:

95	°C	5	min	X35
95	°C	30	sec	
58	°C	40	sec	
72	°C	60	sec	
72	°C	10	min	

PCR program KO:

95	°C	5	min	X35
95	°C	30	sec	
60	°C	30	sec	
72	°C	40	sec	
72	°C	10	min	

Expected fragment size:

wt	118	bp
KOfirst	313	bp

Comments/Additonal information: