

Genotyping protocol

General information:

Strain name	Cnbc:NMRI-conls<+>
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Primers:

Name	Sequence	Primer type
Fw	CATCGAGACCAAGTTTTCTG	
Rev	ACCATGTCGTAGGCACTGAG	

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

	Forward primer	Reverse primer
Mutant	Fw	Rev

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	4	min	X36
94	°C	15	sec	
51	°C	60	sec	
72	°C	60	sec	
72	°C	10	min	

Expected fragment size:

wt	Bp
mutant	Bp

Comments/Additonal information:

PCR followed by an MseI restriction digest. Digesting PCR products with MseI generates a single band of 362 bp.