

Genotyping protocol

General information:

Strain name	R26-rtTA _g /0:tetO-Cpt1atg/0 (R26rtTA-CPT1A)
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Primers:

Name	Sequence	Primer type
R26-rtTA Rv 1	GCGAAGAGTTTGTCTCAACC	R26-rtTA transgene
R26-rtTA Rv 2	GGAGCGGGAGAAATGGATATG	R26-rtTA transgene
R26-rtTA Fw	AAAGTCGCTCTGAGTTGTTAT	R26-rtTA transgene
CPT1A Fw 1	GCACAGCATTGCGGACATGC	CPT1A transgene
CPT1A Rv	CCCTCCATGTGTGACCAAGG	CPT1A transgene
CPT1A Fw2	GCAGAAGCGCGGCCGTCTGG	CPT1A transgene

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

	Forward primer	Reverse primer
R26-rtTA WT allele	R26-rtTA Rv 1	R26-rtTA Fw
R26-rtTA MUT allele	R26-rtTA Rv 2	R26-rtTA Fw
CPT1A WT allele	CPT1A Fw 1	CPT1A Rv
CPT1A MUT allele	CPT1A Fw2	CPT1A 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
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Taq Polymerase 5U/μl	0,250	μl
DNA	1	μl
Final volume	25	μl

PCR program R26-rtTA:

95	°C	5	min	X35
95	°C	30	sec	
59	°C	45	sec	
72	°C	45	sec	
72	°C	10	min	

PCR program TRE-CPT1A:

95	°C	5	min	X35
95	°C	30	sec	
59	°C	45	sec	
72	°C	45	sec	
72	°C	10	min	

Expected fragment size:

R26-rtTA WT allele	650	bp
R26-rtTA MUT allele	300	bp
CPT1A WT allele	294	bp
CPT1A MUT allele	450	bp

Comments/Additonal information:

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