

# Genotyping protocol

## General information:

Strain name	B6.Cg-Tg(Pax8-rtTA2S*M2)1Koes Tg(tetO-Cpt1a,-EGFP)/Cnbc
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## Primers:

Name	Sequence	Primer type
Pax8 Fw	CCATGTCTAGACTGGACAAGA	Pax8-rtTA transgene
Pax8 Rv	CTCCAGGCCACATATGATTAG	Pax8-rtTA transgene
CPT1A Fw1	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
rtTA construction	Pax8 Fw	Pax8 Rv
CPT1A WT allele	CPT1A Fw1	CPT1A Rv
CPT1A MUT allele	CPT1A Fw2	CPT1A Rv

## Reaction mix:

ddH <sub>2</sub> O	18,5	μl
PCR Buffer 15mM MgCl <sub>2</sub>	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 Pax8-rtTA:

95	°C	5	min	X40
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:**

Pax8-rtTA WT allele	No band
Pax8-rtTA mutant allele	595 bp
CPT1A-rtTA WT allele	294 bp
CPT1A-rtTA mutant allele	450 bp

**Comments/Additional information:**

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