

# Genotyping protocol

## General information:

Strain name	STOCK Cdk4tm2.1Bbd/Cnbc
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## Primers:

Name	Sequence	Primer type
pDC1	TCTTTGTGCCTAGTGCGATG	gene-specific
pDCWC	ATGATCACCCAGCTAGTCGTC	gene-specific
pDCKO	TACCAGAGCGTAACCACCTG	gene-specific

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

	Forward primer	Reverse primer
WT allele	pDC1	pDCWC
Mutant allele	pDC1	pDCKO

## 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:

94	°C	1	min	X30
94	°C	60	sec	
63	°C	30	sec	
72	°C	30	sec	
72	°C	10	min	

## Expected fragment size:

Wt allele	640bp
Mutant allele	WT 1300bp KO 450bp

## Comments/Additonal information:

Provider or PubMed ID: 19383288, 20609353