

# Genotyping protocol (detection of mutant animals)

## General information:

Strain name	R26-STOP-GB2
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## Primers:

Name	Sequence	Primer type
G2HA-F	5'-TGGGTTCCGGAGGATTCCAGA-3'	Gene specific
G2HA-R	5'-AGCATAATCAGGAACATCATACGG-3'	Gene specific
		please select one
		please select one

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

	Forward primer	Reverse primer
e.g. wt		
e.g. mut		

## Reaction mix:

G2HA-F (10 $\mu$ M stock)	2,5	$\mu$ l
G2HA-R (10 $\mu$ M stock)	2,5	$\mu$ l
dNTPs (10 mM)	0,5	$\mu$ l
Taq reaction buffer 10x	2,5	$\mu$ l
Taq polimerase	1	$\mu$ l
DNA template	1	$\mu$ l
H2O	15	$\mu$ l
		$\mu$ l
Final volume	25	$\mu$ l

## PCR program:

94	°C	5	min	X35
94	°C	30	sec	
64	°C	30	sec	
72	°C	40	sec	
72	°C	5	min	

## Expected fragment size:

wt		bp
mutant	548	bp

## Comments/Additonal information:

This PCR method allows the identification of mutant mice but it does not distinguish heterozygous from homozygous animals. To this aim, a second PCR reaction (specified below should be performed).

# Genotyping protocol (detection of Wt allele and discriminating heterozygous from homozygous mice)

## General information:

Strain name	R26-STOP-GB2
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## Primers:

Name	Sequence	Primer type
ROSA26-F	5'-TATCAGTAAGGGAGCTGCA -3'	Wild type allele
ROSA26-R	5'-ACCCAGATGACTACCTATCC -3'	Wild type allele

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

	Forward primer	Reverse primer
e.g. wt		
e.g. mut		

## Reaction mix:

ROSA26-F (10 $\mu$ M stock)	2,5	$\mu$ l
ROSA26-R (10 $\mu$ M stock)	2,5	$\mu$ l
dNTPs (10 mM)	0,5	$\mu$ l
Taq reaction buffer 10x	2,5	$\mu$ l
Taq polimerase	1	$\mu$ l
DNA template	1	$\mu$ l
H2O	15	$\mu$ l
		$\mu$ l
Final volume	25	$\mu$ l

## PCR program:

94	°C	5	min	X35
94	°C	30	sec	
62	°C	30	sec	
72	°C	30	sec	
72	°C	5	min	

## Expected fragment size:

wt	300	bp
mutant		bp

## Comments/Additonal information:

This PCR method detects the Wt ROSA26 allele. It amplifies DNA from Heterozygous and Wt animals but not homozygous mutant animals. The combination of this PCR with the method described in the previous page allos the discrimination between heterozygous and homozygous mice.

