Genotyping protocol

General information:

Strain name	Bex3KO

Primers:

Name	Sequence	Primer type
Bex3F-Ext	CCTGTCTAGGACCCCTGTGA	gene-specific
Bex3R-Ext	GCGGGAGTCACAGTATGGAT	gene-specific
Bex3F-Int	AGCCCCCACTCCACACTACT	gene-specific
Bex3R-Int	TGGTGATCGTGGTGGTTAGA	gene-specific

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

	Forward primer	Reverse primer
External PCR	Bex3F-Ext	Bex3R-Ext
Nested internal PCR using 1 :10 dilution of PCR product from the external PCR	Bex3F-Int	Bex3R-Int

Reaction mix:

gDNA (external PCR) // 1 :10 dilution external PCR (nested PCR)	1	µl
10 x buffer w/o Mg2+	2	µl
MgCl2 25 mM	1.2	µl
dNTPs 2mM	1	µl
Forward primer 25 uM	0.4	µl
Reverse primer 25 uM	0.4	µl
Taq polymerase	0.2	µl
H2O	13.8	µl
Final volume	20	µl

PCR program:

External PCR :

95	°C	5	min	
95	°C	30	sec	X 30
55	°C	30	sec	
72	°C	30	sec	
72	°C	10	min	

Nested PCR :

95	°C	5	min	
95	°C	30	sec	X 30
59	°C	30	sec	
72	°C	30	sec	
72	°C	10	min	

Expected fragment size:

wt	682	bp
mutant	535	bp

Comments/Additonal information:

Genotyping the Bex3KO line requires two consecutive rounds of PCRs. First, Bex3F-Ext and Bex3R-Ext primers are used. The product of this first round of PCR does not need to be run. Then, 1 ul of a 1:10 dilution (in H2O) of the previous PCR product is used in the second round of PCR (nested PCR) with primers Bex3F-Int and Bex3R-Int. The expected band sizes are indicated above. Note that this gene is located in chromosome X, so males can never be heterozygous, thus showing only either the WT or the mutant band.