Noggin genotyping by PCR
Protocol adapted on 10/10/12

The Noggin genotyping is done by 2 PCRs, 1 PCR to amplify the wild type gene and 1 to amplify the knockout gene.
Prepare the mix on ice.

PCR mix per sample.

	WT (µl)	KO (µl)
H_2O (Fresh Milli-Q)	6.5	6.5
Buffer	1	1
$MgCl_2$	0.5	0.5
dNTP	1	1
primers 210 x 211	1	
primers 210 x 212	-	1
Enzyme (Taq-Pol.)	0.15	0.15
Template DNA	1,5	1,5
Betaine 5M	2	2

Mix by pipetting

PCR program: 2-step PCR.

95 °C - 2 min

95 °C - 20 seconds
72 °C - 1 min } 32 cycles

72 °C - 3 min
4 °C - ∞

The number of cycles may be increased depending on the quality of DNA (for instance not isolated by the machine)

Expected size:
Around 200 basepairs for the WT PCR (210 x 211)
Around 170 basepairs for the KO PCR (210 x 212)

Results:
Only the WT PCR amplified the fragment → mouse is a wildtype
Only the KO PCR amplified the fragment → mouse is a knockout
Both PCRs amplified the fragments → mouse is a heterozygote

Remarks:
Use buffer, MgCl2 and enzyme from the same kit (Lot#).
Don't forget the control samples. There is a special plate with control samples for al PCRs in fridge 3 in a box (which normally contains 96-Well Optical Reaction Plates for purifying DNA).

PRIMERS:
- primers for WT pcr
F: gcatggagcgctgccccagc
R: gagcagcgagcgcagcagcg

- primers for KO pcr
F: gcatggagcgctgccccagc (same F primer as for WT pcr)
R: aagggcgatcggtgcgggcc

PRODUCTS:
- Buffer, MgCl2 and Taq polymerase: Bioline Biotaq polymerase 2500 units; cat nr BIO-21060
- dNTP: Life technologies 10mM dntp mix; cat nr 18427-013
- Betain: Sigma-Aldrich 5M betain; cat nr B0300-1VL