

PROTOCOL FOR BDNF(NT3/NT3) MOUSE GENOTYPING

Procedure

Genotyping of offspring from BDNF(NT3/NT3) breeding colony is based on PCR.

PCR primers

5' forward primer (PGK) - 5' **ggg aac ttc ctg act agg gg** 3'
3' reverse primer (BL1) - 5' **atg aaa gaa gta aac gtc cac** 3'
3' reverse primer (BL2) - 5' **cca gca gaa aga gta gag gag** 3'

PCR profile

95 °C, 5 min
92 °C, 1 min 33 cycles
56 °C, 1 min
74 °C, 2 min
74 °C, 10 min
4 °C, ∞

PCR mix

10 x PCR Gold buffer (Perkin Elmer)	3.0 µl
MgCl ₂ (25 mM)	1.5 µl
dNTPs (10 mM)	0.5 µl
PGK (20 µM)	0.5 µl
BL1 (20 µM)	0.5 µl
BL2 (20 µM)	0.5 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
ddH ₂ O	<u>21.3 µl</u>
	30 µl

Post-PCR analysis

Load 10 µl of the PCR reaction on a 2.5 - 3% agarose gel, run on max 50V.
Expected results; two bands are given ~ 350 bp for wt and ~ 480 bp for KO.