

PROTOCOL FOR hGFAPprom lacZ line 9 MOUSE GENOTYPING

Procedure

Genotyping of offspring from hGFAPprom lacZ line 9 breeding colony is based on PCR.

PCR primers

5' forward primer (GFAPp fwd) - 5' **cat gtg taa cgg ctt tgt gg** 3'
3' reverse primer (GFAPp rev) - 5' **gga att tgg cga aga atg aa** 3'

PCR profile, HGFAP

95 °C, 10 min

95 °C, 1 min	10 cycles
60 °C, 2 min	
72 °C, 1 min	

95 °C, 1 min	25 cycles
60 °C, 30 s	
72 °C, 1 min	

72 °C, 10 min
4 °C, ∞

PCR mix

10 x PCR Gold buffer (Perkin Elmer)	2.5 µl
MgCl ₂ (25 mM)	1.0 µl
dNTPs (10 mM)	0.5 µl
GFAPp forw. (20 µM)	0.5 µl
GFAPp rev. (20 µM)	0.5 µl
AmpliTaq Gold (5 U / µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	1.0 µl
ddH ₂ O	<u>18.8 µl</u>
	25 µl

Post-PCR analysis

Load 10 µl of the PCR reaction on a 1 % agarose gel, run on 90V.
Expected results; one band is given 376 bp.