PROTOCOL FOR MyHCa-KCNE1 MOUSE GENOTYPING

Procedure

Genotyping of offspring from MyHCa-KCNE1 breeding colony is based on
PCR. Heterozygous males are breed with C57Bl/6N females.

PCR primers

5' forward primer (MyHCa) - 5´ **ccc ggc act ctt agc aaa cct ca** 3´
(Acc.nummer U71441, nt 5222-5244)
3' reverse primer (KCNE1) - 5´ **gga cac ggg cct gga aga cgg cct t** 3´
(Acc.nummer X60457, nt 376-352)

PCR profile, program MYHCA-KCNE1

95 ˚C, 5 min

95 ˚C, 30 s 30 cycles
63 ˚C, 30 s
72 ˚C, 60 s

72 ˚C, 10 min
4 ˚C, ∞

PCR mix

10 x PCR Gold buffer (Perkin Elmer)	3.0 µl
$MgCl_2$ (25 mM)	3.0 µl
dNTPs (10 mM)	0.5 µl
MyHCa (20 µM)	0.5 µl
KCNE1 (20 µM)	0.5 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
ddH_2O	20.3 µl
	30 µl

Post-PCR analysis

Load 10 µl of the PCR reaction on a 1% agarose gel.
Expected results; one band is given ~ 600 bp