

Genotyping protocol

EM:10416

B6.Cg-Ddn^{tm1Ktry}/Kctt

Primers

Dendrin WTR - 5' **agg ttc aag gcc tct cca tt** 3'Dendrin WTL - 5' **gga gga tct cag cgt cca ta** 3'Dendrin KOL - 5' **aat tcc atc aga cct cga cct** 3'

PCR profile

95 °C, 5 min

95 °C, 1 min 30 cycles

55 °C, 1min

72 °C, 1 min

72 °C, 10 min

4 °C, ∞

PCR mix

10 x PCR Buffer II (Life technologies)	3.0 µl
MgCl ₂ (25 mM)	1.8 µl
dNTP mix (25 mM of each nucleotide)	0.24 µl
Dendrin WTR (200 µM)	0.15 µl
Dendrin WTL (200 µM)	0.15 µl
Dendrin KOL (200 µM)	0.15 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
H ₂ O	<u>22.31 µl</u>
	30 µl

Post-PCR analysis

1%- 1.5% agarose gel.

Expected pattern is **mutant: 405 bp****Wt: 574 bp**