

Genotyping protocol

EM:13207

C3HeB/FeJ-Jag1^{Ndr}/Ieg

Primers

Nodder fw	5' GTG TGT TGT AAC TAT GTG CA 3'
Nodder rev	5' GAG TCC CAC AGT AAT TCA GA 3'
Nodder seq	5' GGT CAT ACC TTT GTC ACA GA 3'

PCR profile

94 °C, 5 min	
94 °C, 30 s	30 cycles
58 °C, 45 s	
72 °C, 45 s	
72 °C, 10 min	
4 °C, ∞	

PCR mix

10 x PCR Buffer II (Life technologies)	2.0 µl
MgCl ₂ (25 mM)	1.2 µl
dNTP mix (25 mM of each nucleotide)	0.16 µl
Nodder fw (100 µM)	0.1 µl
Nodder rev (100 µM)	0.1 µl
AmpliTaq Gold (5 U/µl)	0.13 µl
DNA template (~ 0.5 µg tail DNA)	1.0 µl
H ₂ O	<u>15.31 µl</u>
	20 µl

Sequence the PCR product using Nodder seq primer.

Post-PCR analysis

Wt CGT GGA CAC ATC CTG **GGT** GCG GGA TGC ACT

Mut CGT GGA CAC ATC CTG **GTT** GCG GGA TGC ACT