

Genotyping protocol

EM:13213

B6N.Cg-Polg^{tm1.1Lrsn}/Kctt

Primers

PolgA-mut F	5' CTT CGG AAG AGC AGT CGG GTG 3'
PolgA-mut R	5' GGG CTG CAA AGA CTC CGA AGG 3'

PCR profile

94 °C, 5 min	
94 °C, 30 s	
58 °C, 45 s	30 cycles
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
PolgA-mut F (100 µM)	0.1 µl
PolgA-mut R (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

Post-PCR analysis

1.5% agarose gel.
Expected pattern is wt:520bp, mut:720bp