

$\Delta V1rab\Delta/J$

Primer sequence

Wildtype

Forward primer: GAA GTG AGC AGG ACT GAG TA

Reverse Primer: CAT CAG CTG ACA TGC CTA TA

Band size 1442bp

Mutant

Forward primer: GCA CCA CTA CTT CTT CCT CA

Reverse primer: TAG GGG AGG AGT AGA AGG T

Band size 300bp

PCR Reaction (μ l)

H2O	18.07
10x Buffer	2.50
dNTP (2.5mM each)	2.00
Primer 1 (20 μ M)	0.40
Primer 2 (20 μ M)	0.40
HS-Taq Polymerase (5 U/ μ l)	0.13
DNA (<500ng)	1.50
Total volume	25.00

Takara Taq Hot Start Version, Cat. No. R007A

DNA extraction with DirectPCR tail, PEQLAB Biotechnologie GMBH and Proteinase K, Bioline GmbH

PCR condition

95°C 5min

95°C 1min

60°C 1min

72°C 2min

72°C 10min

4°C ∞

} 35x

