

PCR Protocol for strain #30 Figla tm1Dean

Reaction mix

1 μ l DNA
2.5 μ l 2mM dNTPs
2.5 μ l 10x buffer
0.75 50mM MgCl₂
1.25 μ l of each primer (three primers at a concentration of 10pmol/ μ l)
0.15 μ l Taq
14.35 μ l HPLC water
25 μ l Total

Primers

A3 (22mer)
5'-CTGGCCCCTTCGTGGTACCTCC-3'

A4 (21 mer)
5'-GGGCCTGTGAAGGGTCAGCCC-3'

TLR34 (22mer)
5'-CATTTGTCACGTCCTGCACGAC-3'

PCR CONDITIONS

Start with 94 degrees 5 min.

The following for 35 cycles

94 degrees 30 sec

65 degrees 30 sec

72 degrees 1 min

Finish with

72 degrees 10 min

4 degree hold

results when run on 0.7% Agarose Gel

wt allele at approx. 350 bp

null allele just under 500 bp

when the animal is heterozygous there will be an artifact band that is present at approx. 600 bp