ache ^{tf222a}

Nature of the mutation

The *tf222a* allele contains a single G-to-A missense mutation that substitutes Gly 198 with an Arg residue (Downes and Granato, Developmental Biology 270: 232-245, 2004).

Genotyping assay

Genotyping of the *tf222a* allele is based on the ASA assay (**A**llele-**S**pecific **A**mplification) (Newton et al., Nucleic Acids Research 17 (7): 2503-2516, 1989; Kwok et al., Nucleic Acids Research 18 (4): 999-1005, 1990). In this method, a PCR primer set is designed in such a way that the 3'-terminal nucleotide of one primer corresponds to the point mutation with respect to its location in the DNA and base pairing. This nucleotide therefore represents a mismatch for the WT template. Because a single mismatch is typically insufficient to achieve a desired level of discrimination required for allele-specific PCR amplification, additional mismatches are introduced to further increase this discrimination.

To genotype *tf222a*, the ACMM_09 primer carries two mismatches for the WT template (marked in red), and one mismatch for the mutant DNA (underlined). This primer together with ACWW_10 gives rise to PCR amplification only if a sample contains the *tf222a* mutation. No PCR amplification is detected for WT samples. An additional primer set (BAA11 and BAA12) is used as an internal positive control. PCR reaction conditions (e.g. reagent concentrations) have been established empirically and need to be strictly followed for successful *tf222a* genotyping.

Reagent concentrations in the PCR reaction:
dNTP concentration: 60 µM each
Mg^{+2} concentration: 1.5 mM
tf222a–specific primer concentration (ACMM_09 and ACWW_10): 0.4 µM each
control primer concentration (BAA11 and BAA12): 0.08 µM each

***tf222a*-specific primers:**
ACMM_09: 5' GCT GCC CCA GCA CTT TCT AT 3'
ACWW_10: 5' TTC CTC TCT TGA TGT CTA TGA TGG 3'

Control primers:
BAA11: 5' TCT GGA TTT CTG ACT AAC CCC TGC 3'
BAA12: 5' CAG AGT CCA TCA CGA TAC CAG TGG 3'

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PCR program (56_C_30):

1. 94ºC for 3 min
2. 94ºC for 30 sec
3. **56**ºC for **40** sec
4. 72ºC for **40** sec
5. Go to step 2 (above) for 29 cycles
6. 72ºC for 5 min
7. 8.0ºC hold
8. END

Product size: 240 bp for ACMM_09 and ACWW_10
195 bp for BAA11 and BAA12

The 240 bp product is specific for the *tf222a* mutant genomic DNA. This product is not generated for wild-type genomic DNA. The 195 bp product is detected for both mutant and wild-type genomic DNA.

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