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(57) Abstract :
A method for performing a high-fidelity Tetra-Primer Amplification Refractory Mutation System (TETRA-ARMS) multiplex PCR assay is disclosed. The method defies conventional manufacturer guidelines by utilizing a commercial PCR master mix at a functional sub-1× concentration within the final reaction volume. Reducing the master mix concentration below standard specifications strategically limits components such as magnesium ions and dNTPs. This restriction unexpectedly increases primer hybridization stringency, suppressing mispriming events and primer-dimer artifacts without compromising target amplification efficiency. The method provides exceptionally clean genotype resolution in single-tube multiplex allele discrimination assays.

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