Multiplex PCR Assay Using Specific Primers for Species Identification in Meat Products

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Abstract

Recently, Polymerase Chain Reaction (PCR) has been widely used for species authentication, given their simplicity, specificity, and sensitivity. Multiplex PCR (mPCR) assays allow the simultaneous identification and differentiation of several species by using species-specific primers (forward and reverse primers). The present study aims to develop multiplex (heptaplex) PCR using newly designed specific primers for identification of seven commonly consumed meat, namely, beef, goat, sheep, buffalo, chicken, duck, and pork. Multiple-target detection in a single reaction saves both time and analytical costs. However, the design of primer sets used in mPCR assays is more critical and complicated since all biomarkers must be annealed to their respective targets under a single set of PCR conditions. In this study, seven short length biomarkers (73 - 263 bp) were designed for each of cow (106 bp), goat (236 bp), buffalo (138 bp), sheep (263 bp), chicken (161 bp), duck (203 bp), and pig (73 bp) to develop mPCR assay targeting mitochondrial ND5 and cytb genes. After downloading the sequences from the National Center for Biotechnology Information (NCBI) website, the primer3 v.0.4.0 software was used. The designed primers were verified for specificity in-silico against 7 targets and 19 non-target species. The complete sequence matched only with the target species whereas considerable mismatching was observed with other non-targets. The pairwise distances analyzed through the neighbor-joining method reflected enough genetic distances among the studied species, eliminating the possibility of any cross-target amplification and thus confirming the target detection by the developed mPCR assay system.

Keywords: Multiplex PCR, Authentication, Primer design, Mitochondrial gene, Species-identification