Identification of short-length oligonucleotides biomarker for canine species detection using mitochondrial cytochrome b gene

ABSTRACT

Introduction: Stray dogs are still available in certain countries without any offered price and made it as a potential source for adulteration with costly meats for more benefit. Furthermore, human forensic evidences from crime scenes were often integrated with biomaterial of canine origin. Most of the DNA based assay for canine species detection used longer amplicon size (>150 bp) which are not suitable for highly degraded food or forensic sample analysis. Therefore, in this study for development of short length canine specific biomarker, mitochondrial cytochrome b (cytb) gene was targeted using simple PCR assay.

Objective: Detection of canine species using short length DNA biomarker targeting cytb gene.

Methods: The assay targeted a 100-bp fragment of cytochrome b gene using a pair of canine specific primers. The primers specificity were tested under Insilco, as well as in real PCR assay using dog and eight other species DNAs. The consensus 100 bp canine specific site along with cytb sequences of 14 species including dog and human were used for analysis of pair wise distances, construct dendogram and primers mismatch calculation. The stability of the biomarker was tested under commonly used cooking condition and extensive autoclaving state which was known for degradation of target DNA. The sensitivity of the assay was tested using binary admixture composed of dog and most consumed chicken DNA pool.

Results & Discussion: The biomarker was 100% canine specific and successfully amplified 100 bp region of canine cytb gene specific target. It was highly stable and sensitive enough to detect as low as 0.1% (0.02 ng) of canine specific target from admixed DNAs.

Conclusion: The primers provided the shortest DNA biomarker for canine species detection. The shortest amplicon length, high stability and sensitivity offered its potentiality for canine biomaterials determination from food as well as from degraded samples.