

Development of a Multi-Locus DNA Metabarcoding Method for Endangered Animal Species Identification

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Abstract : Objectives: The identification of endangered species, especially simultaneous detection of multiple species in complex samples, plays a critical role in alleged wildlife crime incidents and prevents illegal trade. This study was to develop a multi-locus DNA metabarcoding method for endangered animal species identification. Methods: Several pairs of universal primers were designed according to the mitochondria conserved gene regions. Experimental mixtures were artificially prepared by mixing well-defined species, including endangered species, e.g., forest musk, bear, tiger, pangolin, and sika deer. The artificial samples were prepared with 1-16 well-characterized species at 1% to 100% DNA concentrations. After multiplex-PCR amplification and parameter modification, the amplified products were analyzed by capillary electrophoresis and used for NGS library preparation. The DNA metabarcoding was carried out based on Illumina MiSeq amplicon sequencing. The data was processed with quality trimming, reads filtering, and OTU clustering; representative sequences were blasted using BLASTn. Results: According to the parameter modification and multiplex-PCR amplification results, five primer sets targeting COI, Cytb, 12S, and 16S, respectively, were selected as the NGS library amplification primer panel. High-throughput sequencing data analysis showed that the established multi-locus DNA metabarcoding method was sensitive and could accurately identify all species in artificial mixtures, including endangered animal species *Moschus berezovskii*, *Ursus thibetanus*, *Panthera tigris*, *Manis pentadactyla*, *Cervus nippon* at 1% (DNA concentration). In conclusion, the established species identification method provides technical support for customs and forensic scientists to prevent the illegal trade of endangered animals and their products.

Keywords : DNA metabarcoding, endangered animal species, mitochondria nucleic acid, multi-locus

Conference Title : ICEAS 2022 : International Conference on Endangered Animals

Conference Location : Lisbon, Portugal

Conference Dates : September 20-21, 2022