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Volume 33, Issue 11, 1 November 2016, Pages 1643-1659**Duplex real-time PCR assay using SYBR Green to detect and quantify Malayan box turtle (*Cuora amboinensis*) materials in meatballs, burgers, frankfurters and traditional Chinese herbal jelly powder** (Article)Asing<sup>a</sup>, Ali, E.<sup>ab</sup> , Hamid, S.B.A.<sup>a</sup>, Hossain, M.<sup>a</sup>, Ahamad, M.N.U.<sup>a</sup>, Hossain, S.M.A.<sup>a</sup>, Naquiah, N.<sup>a</sup>, Zaidul, I.S.M.<sup>c</sup> <sup>a</sup>Nanotechnology and Catalysis Research Center (NANOCAT), University of Malaya, Kuala Lumpur, Malaysia<sup>b</sup>Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, Kuala Lumpur, Malaysia<sup>c</sup>Department of Pharmaceutical Technology, Faculty of Pharmacy, International Islamic University, Kuantan, Malaysia

## Abstract

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The Malayan box turtle (*Cuora amboinensis*) (MBT) is a vulnerable and protected species widely used in exotic foods and traditional medicines. Currently available polymerase chain reaction (PCR) assays to identify MBT lack automation and involve long targets which break down in processed or denatured tissue. This SYBR Green duplex real-time PCR assay has addressed this research gap for the first time through the combination of 120- and 141-bp targets from MBT and eukaryotes for the quantitative detection of MBT DNA in food chain and herbal medicinal preparations. This authentication ensures better security through automation, internal control and short targets that were stable under the processing treatments of foods and medicines. A melting curve clearly demonstrated two peaks at  $74.63 \pm 0.22$  and  $78.40 \pm 0.31^\circ\text{C}$  for the MBT and eukaryotic products, respectively, under pure, admixed and commercial food matrices. Analysis of 125 reference samples reflected a target recovery of 93.25–153.00%, PCR efficiency of 99–100% and limit of detection of 0.001% under various matrices. The quantification limits were 0.00001,  $0.00170 \pm 0.00012$ ,  $0.00228 \pm 0.00029$ ,  $0.00198 \pm 0.00036$  and  $0.00191 \pm 0.00043$  ng DNA for the pure meat, binary mixtures, meatball, burger and frankfurter products, respectively. The assay was used to screen 100 commercial samples of traditional Chinese herbal jelly powder from eight different brands; 22% of them were found to be MBT-positive ( $5.37 \pm 0.50$ – $7.00 \pm 0.34\%$  w/w), which was reflected through the Ct values ( $26.37 \pm 0.32$ – $28.90 \pm 0.42$ ) and melting curves ( $74.63$ – $78.65 \pm 0.22^\circ\text{C}$ ) of the amplified MBT target (120 bp), confirming the speculation that MBT materials are widely used in Chinese herbal desserts, exotic dishes consumed with the hope of prolonging life and youth. © 2016 Informa UK Limited, trading as Taylor & Francis Group.

## Author keywords

herbal jelly powder limits of detection and quantification Malayan box turtle protected species SYBR Green real-time PCR

## Indexed keywords

Engineering controlled terms: Assays Binary mixtures Chains Chemical contamination Melting

Compendex keywords: Limits of detection Malayan box turtle Medicinal preparation protected species  
Quantification limit Quantitative detection Real-time PCR Real-time PCR assay

Engineering main heading: Polymerase chain reaction

EMTREE drug terms: cytochrome b RNA 18S DNA herbaceous agent organic compound powder  
SYBR Green IMetrics  [View all metrics >](#)1 Citation in Scopus  
75th Percentile  
0.70 Field-Weighted  
Citation ImpactPlumX Metrics Usage, Captures, Mentions,  
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## Cited by 1 document

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Hossain, M.A.M. , Ali, Md.E. , Sultana, S.  
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(2016) *PLoS ONE*

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EMTREE medical terms:

Article Chinese herb food chain food processing limit of detection  
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## Molecular Sequence Numbers:

GENBANK,

AP003428(submitted), EU755253(submitted), KF907307(submitted), KM612279(submitted), KP168712(submitted), KP269069(submitted), KR059217(submitted), KU050703(submitted), NC001700(submitted), NC001941(submitted), NC001960(submitted), NC002081(submitted), NC006295(submitted), NC006880(submitted), NC007579(submitted), NC011573(submitted), NC012374(submitted), NC012670(submitted), NC016005(submitted)

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cytochrome b, 9035-37-4; DNA, 9007-49-2;

DNA; Drugs, Chinese Herbal; Organic Chemicals; Powders; SYBR Green I

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