

论文

中药材龟甲及原动物的高特异性PCR鉴定研究

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摘要:

目的:建立一种简便、实用的龟甲药材DNA 分子鉴定方法。方法:根据22 种亚洲产龟类的线粒体12SrRNA 基因片段序列,设计一对专用于鉴定中药材龟甲原动物乌龟的鉴别引物,用该对引物扩增从乌龟和其他18 种龟共48 个样品的DNA 模板。结果:在72℃的复性温度下进行PCR,4 个乌龟的模板DNA 均得到约180 bp 的阳性扩增带,而其他各龟的模板DNA,在同样条件下无扩增产物,用这对鉴别引物经一次PCR 反应便可准确地鉴定受试原动物是否为乌龟。同法对江苏省药品检验所提供的17 块样品龟甲进行了鉴定,结果表明只有4 块样品为正品,其余皆为伪品,与性状鉴定和DNA序列分析鉴定结果完全一致。结论:所设计的鉴别引物对乌龟有高度特异性,所配制的龟甲药材鉴定试剂盒可在龟甲药材鉴定中使用。

关键词: 龟甲 乌龟 鉴别引物 PCR鉴定

STUDY ON HIGHLY SPECIFIC DIAGNOSTIC PCR OF THE TRADITIONAL CHINESE MEDICINE TORTOISE PLASTRON AND ITS ORIGINAL ANIMALS

Liu Zhongquan Wang Yiquan; Zhou Kaiya; Yang Xuegan; Cao Lin and Liu Wuxia

Abstract:

AIM: To develop a convenient and effective method for the identification of tortoise plastron. METHODS: Based on the sequence of mitochondrial 12S rRNA gene fragment of 22 species of Asian turtles, a pair of highly specific primers were designed for distinguishing Reeves' turtle (*Chinemys reevesii*), the original animal of tortoise plastron, from other turtles. The primers were employed to amplify the DNA templates extracted from *Chinemys reevesii* and 18 other species of turtles that amounted to 48 samples. RESULTS: An about 180 bp DNA fragment was amplified respectively from 4 individuals of *Chinemys reevesii* in PCR with anneal temperature at 72℃, whereas no any DNA fragment was amplified from other turtle samples under the same reaction condition. *Chinemys reevesii* could be clearly distinguished from others by PCR reaction with the highly specific primers. Seventeen 'tortoise plastrons', provided by the Jiangsu Institute for Drug Control, were also identified by the highly specific PCR with the primers in the present study. The results indicated that only 4 samples were the shells of *Chinemys reevesii* specified in *Pharmacopeia of the People's Republic of China* and the others were substitutes, which was consistent with the conclusion of authentication based on the morphological and DNA sequence analyses. CONCLUSION: The primers designed in the present study were highly specific for *Chinemys reevesii*. They could be used as key components in the tortoise plastron identification kit.

Keywords: *Chinemys reevesii* highly specific primer diagnostic PCR tortoise plastron

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