中国中药杂志
China Journal of Chinese Materia Medica

基于双向位点特异性PCR的金银花真伪鉴别方法研究

投稿时间： 2012-06-06 责任编辑： 点此下载全文

引用本文：蒋超,张雅华,陈敏,袁媛,林淑芳,吴志刚.基于双向位点特异性PCR的金银花真伪鉴别方法研究[J].中国中药杂志,2012,37(24):3752.

DOI：10.4268/cjcmm20122415

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基金项目:国家自然科学基金项目(81001605);中药制药过程新技术国家重点实验室开放基金项目(SKL2010M0101)

中文摘要:目的:筛选获得金银花真伪鉴别SNP位点并建立双向位点特异性PCR方法,用于鉴别金银花和其常见伪品以及两者的混杂品。方法:通过对GenBank收录的忍冬属植物叶绿体*trnL-trnF*序列进行对比分析,获得金银花真伪鉴别SNP位点;并依据该SNP位点设计特异性引物,对84份金银花基原植物及其市售饮片、伪品进行双向位点特异性PCR扩增,并根据真伪品的特异性条带进行金银花药材鉴别。结果:退火温度为61 ℃时,正品均出现468 bp的条带,红腺忍冬、华南忍冬、灰毡毛忍冬、黄褐毛忍冬、水忍冬、金银忍冬、郁香忍冬、新疆忍冬、繁果忍冬等9个伪品均出现324 bp的条带,在正品DNA中掺入5%以上伪品时同时出现正品和伪品条带。结论:双向位点特异性PCR可以鉴别金银花真伪品及两者的混杂样品。

中文关键词:双向位点特异性PCR 金银花 分子鉴定

Authentication of *Lonicera japonica* using bidirectional PCR amplification of specific alleles

Abstract:Objective: To identify SNP in flos Lonicerae, and authenticate *Lonicera japonica* from its adulterants and the mixture by using bidirectional PCR amplification of specific alleles (Bi-PASA). **Method:** SNP of *L. japonica* and its adulterants was identified by using ClustulW to align trnL-trnF sequences of the Lonicera genus from GenBank database. Bi-PASA primer was designed and the PCR reaction systems including annealing temperature optimized. Optimized result was performed in 84 samples to authenticate *L. japonica*, its adulterants and the mixture. **Result:** When the annealing temperature was 61 ℃, DNA from *L. japonica* would be amplified 468 bp whereas PCR products from all of the 9 adulterants were 324 bp. The established method also can detect 5% of intentional adulteration DNA into *L. japonica*. **Conclusion:** The Bi-SPASA could authenticate *L. japonica* from its adulterants and the mixture.

keywords:bidirectional PCR amplification of specific alleles *Lonicera japonica* molecular authentication

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