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Review Article

DNA identification technology: a powerful tool for the quality control of Chinese medicinal drugs

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Abstract: DNA identification technology has been widely utilized in clinical diagnosis of pathogenic microorganisms and genetic diseases. During the past ten years, it has also been incorporated into the Chinese medicinal drugs industry to aid in the authentication of Chinese materia medica (CMM) and raw materials in Chinese patent medicines (CPM). This paper presents an overview of the history behind the development of DNA testing methods for Chinese medicinal drugs, as well as our research team's investigations into authentication of CMM, raw material authentication in CPM, quantitative analysis of Chinese medicinal drugs through DNA quantification, and optimization of DNA testing methods for Chinese medicinal drugs. We also look forward to communicating and collaborating with more institutions to further contribute to the quality control of Chinese medicinal drugs.

Keywords: DNA identification; DNA quantification; Chinese medicinal drugs; Chinese materia medica; Chinese patent medicines

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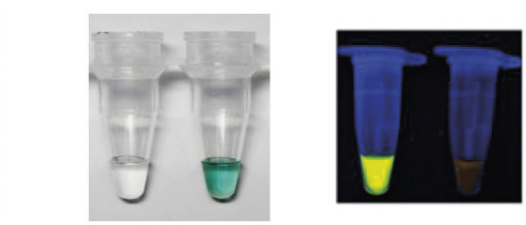
Introduction

Traditional Chinese medicine (TCM) is a treasure of the Chinese nation, which has been protecting the health and happiness of the Chinese people for thousands of years, making significant contributions to the prosperity and strength of the Chinese nation. Chinese medicinal drugs serve as the material foundation for the treatment of diseases in traditional Chinese medicine. The quality of these medicinal drugs is crucial for the efficacy and survival of TCM. Recently, the notion of "traditional Chinese medicine will perish due to the quality of Chinese medicinal drugs" and the idea that "the formula is not working" has appeared. News articles of misuses and adulterations of Chinese medicinal drugs have been reported, such as using apple peel to mimic Isatidis Radix,[1] using cowhide or pig skin

instead of donkey skin to produce Asini Corii Colla,[2] or using toxic Aristolochiae Fructus as Akebiae Caulis has led to severe renal failure in patients.[3] Therefore, establishing more scientific Chinese medicinal drugs quality standards is an important issue for both academia and industry. In order to solve the problem of authenticity and adulteration detection of Chinese materia medica (CMM) and raw materials in Chinese patent medicines (CPM), we have introduced DNA molecular identification technology into the quality control system of Chinese medicinal drugs, and have been exploring and researching for more than ten years, mainly achieving the following four aspects of research progress.

1. DNA identification of Chinese materia medica

The phenomenon of adulteration in CMM is very common, especially in some precious and fine medicinal materials with high economic value. Our research group introduced specific nucleic acid detection technology into the quality control system for CMM to solve the problem of authenticity identification of CMM. So far, we have established colorimetric detection method for Ginseng Radix et Rhizoma,[4] Codonopsis Radix,[4] Croci Stigma,[5] Paridis Rhizoma,[6,7] Pinelliae Rhizoma,[8] Fritillariae Cirrhosae Bulbus,[9] Cordyceps, Asini Corii Colla, Notoginseng Radix et Rhizoma and other easily adulterated medicinal materials. For each medicine, DNA extraction methods were developed and specific amplification primers were designed according to their authentic and counterfeit DNA sequences. PCR or some novel isothermal amplification techniques were used to amplify species-specific DNA and colorimetric detection method for each medicine was established using color reagents. These detection methods were also developed into detectionkits (Figure 1). The related research results have been adopted by enterprises such as the Taiji Group Chongqing Fuling Pharmaceutical, Taiji Group Gansu Tianshui Xihuang Ajiao, and the Sichuan Yuzhi Pharmaceutical to ensure the authenticity of the medicinal materials purchased by them, so as to ensure the authenticity and reliability of the raw materials used in the CPM.



Colorimetric identification of Chinese materia medica

Chinese materia medica DNA testing kit

Figure 1 Colorimetric identification of Chinese materia medica and Chinese materia medica DNA testing kit

2. DNA identification of raw materials in Chinese patent medicines

The adulteration of Chinese herbal medicines has been a long-standing phenomenon, but the adulteration of raw materials in CPM is more common and more difficult to detect. Our research group has developed a DNA column purification and recrystallization technology to address the issue of extracting DNA from complex CPM. This new DNA extraction method, used in combination with PCR technology, allows for the precise identification of the raw material DNA in CPM from various dosage forms, such as pills, tablets, oral liquids and injections. The most representative research works include the DNA identification of *Panax ginseng* in Shengmai Yin (Renshen fomula),[4] *Codonopsis pilosula* in Shengmai Yin (Dangshen fomula),[4] *Panax ginseng* in Shengmai injection and *Pineilia ternata* in Huo Xiang Zheng Qi Shui,[4] *Crocus sativus* in Ershiwuwei Shanhu Wan.[10] In addition, we have applied the functional nucleic acids based visualization chip and visualization fluorescent dyes to report the amplification products, so that the detection results can be presented with color changes visible to the naked eye, realizing the visualization detection of raw materials in CPM (Figure 2).[4]

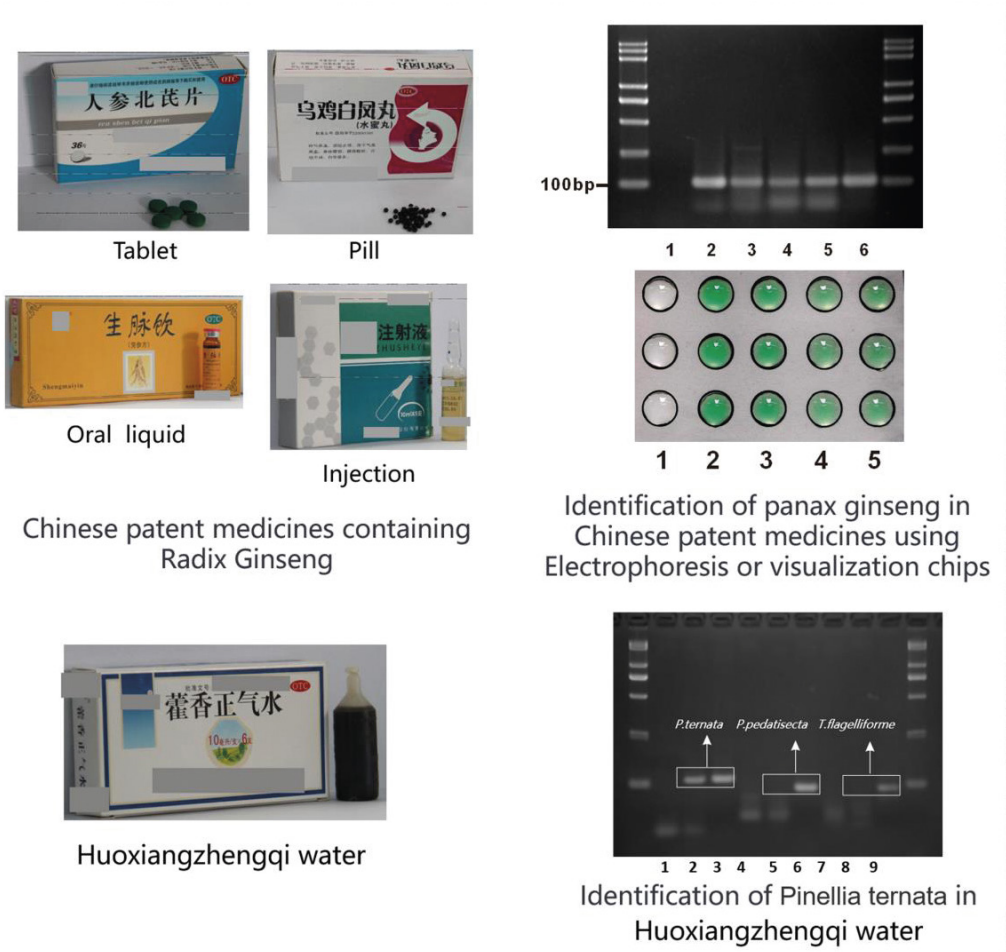
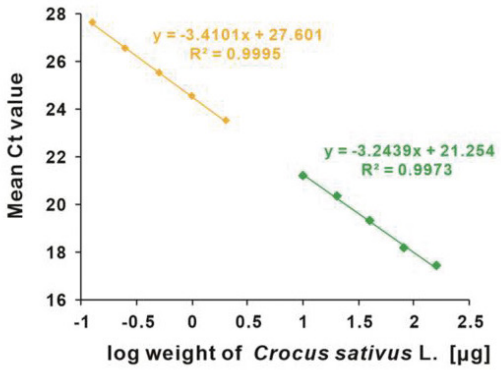


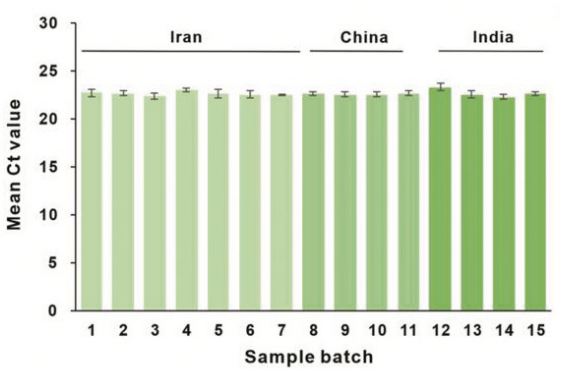
Figure 2 Photos of Chinese patent medicines and DNA identification results of raw materials in Chinese patent medicines

3. Quantitative detection of medicinal materials in crude drugs or Chinese patent medicines with real-time polymerase chain reaction (PCR)

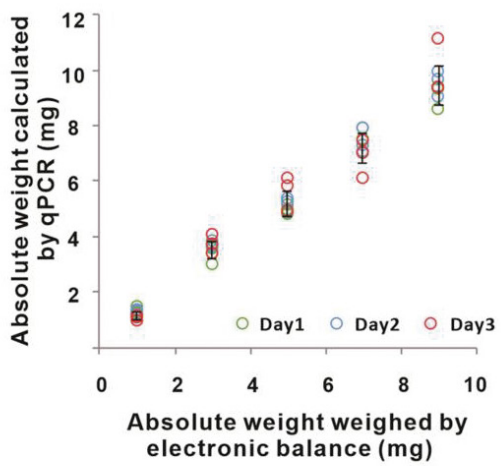
These two works solved the question of "whether it is adulterated", but not "how much it is adulterated". In this regard, our research team studied the correlation between DNA content and herb weight by analyzing a series of saffron herbs of different weights, establishing for the first time a linear relationship between the two. This result enabled us to use DNA content as an effective way to quantify herbal substances. We have also determined the universality of this method by determining the DNA content of herbs from different origins and quantitatively analyzing different proportions of adulterated herbs. This method was then applied to the quantitative analysis of saffron in the Tibetan Patent medicine Ershiwuwei Shanhu Wan (Figure 3).[10]



The linear relationship between the DNA content and the weight of croci stigma



No significant difference in DNA content of croci stigma from different origins



Test results of different proportions of adulterated materials

Sample ID	original (mg/10 mg)	Added (mg)	Found (mg)	△m (mg)	recovery (%)
1	0.04	0.50	0.60	0.56	111.96
2	0.12	0.50	0.69	0.57	113.67
3	0.16	0.50	0.71	0.55	109.91

Determination of saffron DNA content in Chinese patent medicines

Scientific Reports. 2017,7 (1):4774.

Figure 3 Content determination of saffron DNA in Croci Stigma and Ershiwuwei Shanhu Wan

Additionally, we developed quantitative PCR methods for Cordycep, Pinelliae Rhizoma and Pinelliae Rhizoma aqueous decoction in order to achieve a more specific quantitative analysis of the aforementioned herbs.[11] Related research work has been highly evaluated by domestic and foreign experts including researcher Ma Shuangcheng (Director of the Institute of Chinese Medicine and Ethnic Medicine Testing and Certification, China Academy of Food and Drug Control).[12] Moreover, these research results have also been adopted by relevant companies to apply to the quality evaluation of their products.

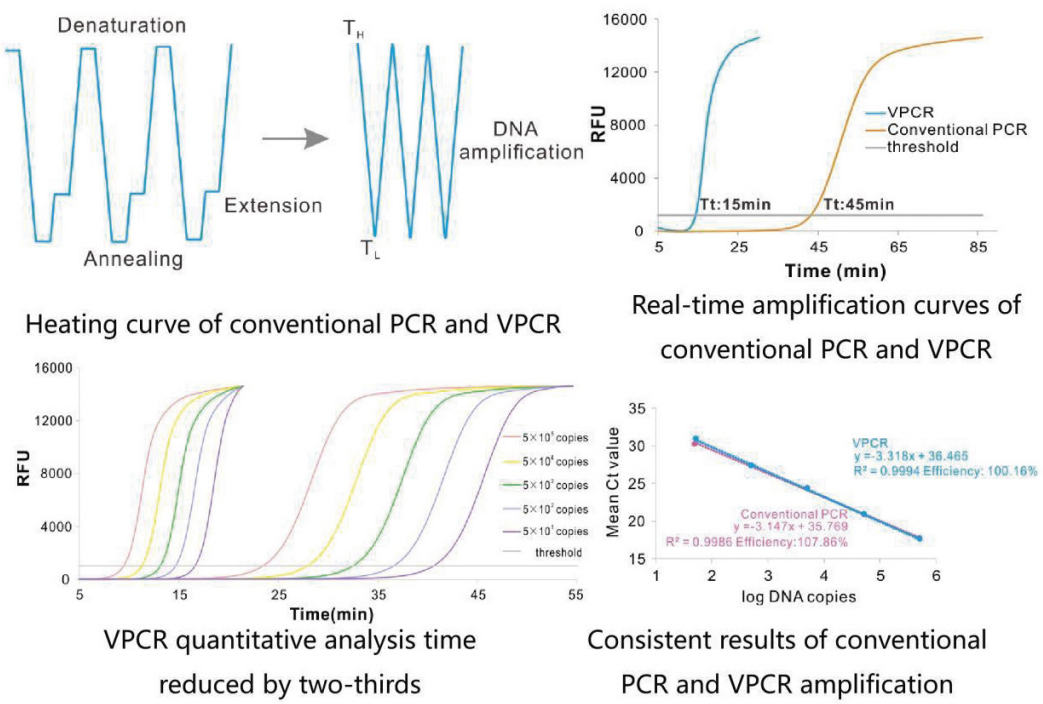
4. Further optimization of DNA detection methods for Chinese medicinal drugs

After establishing a DNA-based system for qualitative and quantitative analysis of CMM and raw materials in CPM, we realized that: the Chinese medicinal drugs DNA testing system established on the basis of traditional molecular biology experiments has some disadvantages such as complicated operation, low throughput and time consuming, proving difficult to implement in practical application and not suitable for the testing of large numbers of samples in Chinese medicinal drugs enterprises and testing institutions. Therefore, we proposed to establish a rapid and high-throughput detection platform suitable for Chinese medicinal drugs DNA detection by studying three aspects - rapid extraction of DNA, rapid amplification of DNA, and real-time detection of amplification products.

In terms of DNA extraction, the traditional column-based nucleic acid extraction method requires the use of a high-speed centrifuge, which is complicated and time-consuming to operate; the fully automated nucleic acid extractor based on the magnetic bead method is also time-consuming and costly. Thus, we switched our research strategy and studied the effects of various secondary metabolites on the rapid extraction of DNA according to the characteristics of different Chinese medicinal drugs themselves, and developed rapid direct lysis extraction reagents for various Chinese medicinal drugs, including Paridis Rhizoma, Cordyceps, Fritillariae Cirrhosae Bulbus, Saffron, Ginseng Radix et Rhizoma, Codonopsis Radix, Notoginseng Radix et Rhizoma. These reagents can directly and rapidly lyse the samples, so that the sample DNA can be released rapidly and the lysis products can be directly used for real-time fluorescence PCR detection, which lays the foundation for automated operation.

In terms of DNA amplification, PCR is the most widely used technique for DNA amplification. It was awarded the Nobel Prize in Chemistry in 1993.[13] In DNA testing of Chinese medicinal drugs, PCR technology is used to amplify the target DNA; however, traditional PCR amplification can take up to several hours to complete. To reduce the time of nucleic acid amplification, researchers and instrument development companies have developed a variety of new PCR instruments that are based on increasing the temperature rise and fall rates through changes in the heating and cooling methods. The various novel rapid PCR instruments reported have mostly been at the basic research stage, still some distance away from commercial application. Through extensive practice, our research team found that PCR does not need to dwell at three temperature points as described in textbooks, but instead can be completed by providing a dynamic variable temperature environment, enabling the reaction material to continuously shift between high and low temperature points. This decreases the amplification time of nucleic acids to one-third of the original time on conventional PCR equipment. We refer to this technique as "VPCR", based on the repetitive "V" shape of the temperature and time curve.

Through a series of methodological studies, we found that VPCR, a dynamic heating method, does not sacrifice amplification efficiency per cycle and was able to reduce the reaction time of PCR from approximately 1 hour to less than 20 minutes with existing nucleic acid amplification devices, while maintaining comparable sensitivity and accuracy to conventional PCR (Figure 4). We also set a record of 8 minutes to complete PCR amplification on a common PCR instrument.[14] Additionally, the method also applied for patent protection (CN108642147A). Subsequently, based on the findings of this study, we established an ultra-fast real-time fluorescence - VPCR amplification assay system for a variety of Chinese herbal medicines such as Pinelliae Rhizoma,[8,15] Saffron, Berberis Cortex and Phellodendri Chinensis Cortex; and successfully shortened the amplification time of Chinese herbal DNA from about 1 hour to less than 20 minutes. To streamline the operation further, we built an automated high-throughput assay platform for the DNA of CMM and CPM using a fully automated pipetting workstation and a real-time fluorescence PCR instrument. This platform utilizes a fully automated pipetting workstation to complete the DNA extraction and build the amplification system, offering rapid extraction of nucleic acids from 96 samples in 5-8 minutes, full sample amplification and detection in 20 minutes, and automated detection of Chinese medicinal drugs DNA in 30 minutes.



Theranostics. 2019,9 (6):1572.

Figure 4 VPCR principle and quantitative analysis results

Summary

In summary, our research team has applied nucleic acid detection technology to authenticate and detect the adulteration in Chinese medicinal drugs. Through a series of original technologies and technology integration, we have developed DNA detection-based qualitative and quantitative analysis technology for Chinese medicinal drugs. We have also developed a rapid nucleic acid amplification method and built a laboratory high-throughput detection platform for both CMM and raw materials in CPM. In fact, the British Pharmacopoeia also employs DNA molecular identification techniques for herb authentication, primarily utilizing DNA barcoding methods. DNA barcoding involves amplifying and sequencing a specific region of DNA using general primers, and then comparing the obtained sequence to reference databases for species identification. The main distinction of our methods and DNA barcoding is the use of species-specific primers, enabling identification based on amplification products without the need for sequencing. This avoids the use of costly and time-consuming sequencing instruments, thereby reducing the cost and time required for detection. Nowadays, we are continuing our efforts to establish DNA identification methods for various species of Chinese medicinal drugs, striving to find innovative methodologies to make DNA testing of Chinese medicinal drugs more efficient, faster, and accurate. We also hope to see more international collaborations and exchanges in the future.

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บทความปริทัศน์

เทคโนโลยีการตรวจพิสูจน์ทาง DNA: เครื่องมืออันทรงประสิทธิภาพในการควบคุมคุณภาพของยาสมุนไพรจีน

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บทคัดย่อ: เทคโนโลยีการตรวจพิสูจน์เอกลักษณ์ทางดีเอ็นเอ ได้ถูกนำมาใช้อย่างกว้างขวางในด้านการตรวจวินิจฉัยทางคลินิกในโรคที่มีสาเหตุมาจากเชื้อจุลินทรีย์ก่อโรคและโรคที่มาจากพันธุกรรม ในช่วงทศวรรษที่ผ่านมาเทคโนโลยีดังกล่าวก็ได้ถูกนำไปใช้ในอุตสาหกรรมยาสมุนไพรจีนโดยช่วยในเรื่องการพิสูจน์ความแท้ของตัวยาสมุนไพรจีนพร้อมใช้หรือวัตถุดิบในยาสำเร็จรูปแผนจีน บทความนี้นำเสนอภาพรวมของประวัติความเป็นมาของการพัฒนาวิธีการทดสอบเอกลักษณ์ทางสารพันธุกรรมดีเอ็นเอยาสมุนไพรจีน รวมถึงการสำรวจติดตามของทีมนักวิจัยในการพิสูจน์ความแท้ของตัวยาสมุนไพรจีนพร้อมใช้และวัตถุดิบที่ใช้ในยาสำเร็จรูปแผนจีน การวิเคราะห์เชิงปริมาณของยาสมุนไพรจีนโดยผ่านวิธีการหาปริมาณด้านดีเอ็นเอ และการวิจัยเพิ่มประสิทธิภาพด้านการตรวจพิสูจน์เอกลักษณ์ทางดีเอ็นเอของยาสมุนไพรจีน ทางคณะวิจัยหวังว่าจะมีการสื่อสารติดต่อและร่วมกันทำงานกับสถาบันหรือหน่วยงานอื่นๆ มากยิ่งขึ้น เพื่อเป็นส่วนหนึ่งที่ก่อให้เกิดการควบคุมคุณภาพของยาสมุนไพรจีนต่อไป

คำสำคัญ: การตรวจพิสูจน์ทางดีเอ็นเอ; การหาปริมาณดีเอ็นเอ; ยาสมุนไพรจีน; ตัวยาสมุนไพรจีนพร้อมใช้; ยาสำเร็จรูปแผนจีน

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文献综述

DNA 分子鉴定技术：中药检测的利器

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摘要： DNA 分子鉴定技术一直被广泛应用于临床病原微生物、遗传疾病等检测，在过去十余年间，也被引入中药行业，用于解决中药材以及中成药中原料药真伪鉴定和掺伪检测的难题。本文介绍了发展中药 DNA 检测方法的历史背景以及本研究团队在中药材的快速真伪鉴定、中成药中原料药的真伪鉴定、基于基因检测的中药定量分析、中药 DNA 检测方法学优化所开展的研究及其相关应用。我们期待能与更多的机构开展合作与交流，为中药的真伪鉴定和质量控制贡献力量。

关键词： DNA 分子鉴定；DNA 定量；中药；中药材；中成药

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